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A preliminary study on effects of subanesthetic doses of preemptive ketamine given prior to premedication on total intravenous anesthesia for short- to medium-term surgical procedures in horses

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Abstract: This study was conducted to determine the effects of subanesthetic doses of ketamine given prior to premedication on the quality of anesthesia, recovery, and postoperative pain in horses. Eighteen horses were randomly recruited into three equal groups, S, LK, and HK, wherein saline, ketamine at 0.2 mg/kg body weight (bwt), and ketamine at 0.4 mg/kg bwt were given, respectively, i.v. for 30 min as a continuous rate infusion (CRI). Horses were premedicated with xylazine at 1 mg/kg bwt i.v. and butorphanol at 0.05 mg/kg bwt i.v. after 30 min. Anesthesia was induced using ketamine and midazolam and was maintained with ketamine at 2 mg/kg/h as CRI, and bolus doses of thiopental 5% solution was given i.v. whenever necessary. Preemptive ketamine infusion clinically enhanced the quality of sedation and enabled smooth induction with significantly ($P < 0.05$) higher sedation and postinfusion ataxia and shorter down-time ($P < 0.05$) in group HK. Physiological, hematological, serological, and vital parameters remained within normal limits. All the horses recovered well without any adverse effects and stood in less than 2 h after surgery. Fluctuation in pain scores at 1 h and 2 h after the end of surgery was minimum in group HK.

Key words: Horse, general anesthesia, preemptive analgesia, subanesthetic dose, ketamine

1. Introduction

A balanced, multimodal approach to anesthesia and analgesia has become the standard for quality patient care in equine surgery. Ketamine is a potent noncompetitive antagonist at *N*-methyl-D-aspartate (NMDA) receptors in the spinal cord that are thought to be responsible for the wind-up, i.e. an exaggerated painful response to relatively innocuous stimuli following a primary injury, and hence it is an effective analgesic (1). Ketamine may also produce analgesia by interaction with opioid receptors in the central nervous system (2). It also contributes to balanced anesthesia by providing intrinsic analgesia during surgery, reduces the drug requirements for maintenance, and enhances recovery during the postoperative period (3,4). Subanesthetic doses of ketamine have been used to provide analgesia in awake and anesthetized horses effectively (5) and to augment sedation along with xylazine and butorphanol in standing anesthesia without any adverse side effects (6). Studies investigating the use of a subanesthetic dose of ketamine at 0.6 mg/kg/h have been shown to enhance analgesia in chronic laminitis (7). Much evidence was obtained from studies where subanesthetic doses of ketamine at 0.8 mg/kg/h were administered in awake horses for more than 6 h without any adverse effects, suggesting that ketamine has marked safety for prolonged infusion in awake horses (8,9). In addition to its analgesic properties, ketamine is well documented to mitigate the stress response to surgical procedures (10) and to have antiinflammatory properties (11). Ketamine continuous rate infusion (CRI) is administered as an adjunct to general anesthesia to maintain and increase anesthetic depth (12). Investigations on CRIs of ketamine in horses have shown minimal cardiovascular depression, preserved laryngeal protective reflexes, and maintained ventilation better than many commonly employed anesthetic or analgesic agents (13,14).

The hypothesis of this investigation was that preoperative administration of subanesthetic doses of

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ketamine 30 min prior to premedication provides better surgical analgesia, enhances sedation, and enables smooth anesthetic induction. The aim of the study was to compare the quality of sedation, quality of induction, quality of surgical anesthesia, quality of anesthetic recovery, and degree of postoperative pain experienced by horses following the use of subanesthetic doses of ketamine as an intravenous (i.v.) infusion administered nearly 30 min before premedication in conventional clinical settings for short- to medium-term surgical procedures. The secondary aim was to evaluate any differences in surgical stress and inflammation in addition to physiological variables and other drug requirements during total i.v. anesthesia (TIVA) in equines. To the best of the authors' knowledge, studies on subanesthetic doses of ketamine as an i.v. infusion given well before premedication along with TIVA in horses have not been reported.

2. Materials and methods

The study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest, and Climate Change, Government of India, as per F. No. 25/33/2016-CPCSEA, and informed consent was obtained from the owner of each horse before any drugs were given.

2.1. Horse details

Eighteen client-owned horses graded ASA 1 or 2 by the physical status classification system of the American Society of Anesthesiologists (15), weighing 265.83 ± 15.3 kg (mean ± SE) and belonging to either sex, referred for surgical procedures requiring nearly 1 h were included in the study. A power calculation was not carried out prior to the start of the study because of limited preliminary data on which such an analysis can rely. Moreover, data collection was bound by the number of eligible horses that were presented to the clinic during the period of the study in order to make appropriate statistical comparisons as horses with severe respiratory, renal, cardiac, or gastrointestinal conditions; pregnancy or lactation; and history of treatment with analgesics were excluded.

2.2. Preemptive ketamine infusion, premedication, and induction

The selected 18 horses were randomly divided into three groups, designated as group saline (S), low ketamine (LK), and high ketamine (HK). The horses were restrained in stocks before the start of any anesthetic administration. The jugular area was clipped and shaved for the placement of a catheter. Adequate time (3–4 h) was allowed for acclimatization of the horse to the environment. Care was taken to avoid noise disturbances during the entire protocol. Body weight measurements were done using a standard estimation formula: body weight (kg) = [girth $(cm)^2$ × length (cm)]/11877 (16). The animals were evaluated by a thorough physical examination that included heart rate (HR), respiratory rate (RR), rectal temperature (RT), gut sounds, color of mucous membrane, and capillary refill time (CRT), along with complete blood count and serum biochemistry. The general behavior of the horses was observed and initial overall pain score were assessed, and the base values for different parameters were recorded before any drugs were given.

In horses of groups LK and HK, preemptive i.v. ketamine (Ketamax 50, Troikaa Pharmaceuticals Ltd., Dehradun, Uttarakhand, India) at 0.2 mg/kg body weight (bwt) and 0.4 mg/kg bwt, respectively, was given along with i.v. normal saline (NS, Nirma Limited, Gujarat, India) for 30 min as a CRI through a 14-gauge jugular venous catheter that was placed into the jugular vein along with a three-way stopcock and fixed onto the neck using secure tape. For the horses of group S, normal saline alone was administered without adding the test drug. During and after preemptive ketamine infusion, the animals were continuously monitored for any abnormal behavior and variations in routine clinical parameters. Ataxia after cessation of preemptive ketamine infusion was scored as 1 (no ataxia), 2 (very mild, slight loss of balance when turning, otherwise steady), 3 (mild, unstable when turning, occasionally staggering when walking), 4 (moderate, frequent staggering when walking, wide-base stance), 5 (moderately severe, needs assistance to prevent falling), or 6 (severe, falls despite assistance) (17). All the assessments and observations were performed by a single blinded observer throughout the study. The observer was unaware of the protocol on subanesthetic doses of ketamine used in each case. Blinding was made complete as ketamine was administered as an i.v. infusion mixed along with normal saline solution.

Thirty minutes after the cessation of infusion (lag period), the animals were administered an $alpha_2$ adrenoceptor agonist, xylazine (Xylaxin, Stanex Drugs and Chemicals Pvt. Ltd., Hyderabad, Andhra Pradesh, India), at 1 mg/kg bwt, i.v., immediately followed by an opioid, butorphanol (Butodol-2, Neon Laboratories Limited, Mumbai, Maharashtra, India), at 0.05 mg/kg bwt, i.v., through the jugular venous catheter using separate syringes. After administration of preanesthetic drugs, horses were left calm to allow the onset of effects of the drugs. Quality of sedation after premedication was scored as 1 (calm, relaxed, no restraint required, minimally responsive to environmental stimuli, reluctant to move), 2 (no restraint required, relaxed, infrequent responses to environmental stimuli, easily walked without problems), 3 (minimal restraint required, interested in environmental stimuli, reactive to noise and sudden movements), or 4 (unsatisfactory, minimal or no signs of sedation, nervousness or apprehension requiring additional sedative

administration) (18). Down-time (min) was recorded in preemptive ketamine-infused horses as the time from the preanesthetic drug administration until the horse achieved recumbency. The recumbent animals were secured on the operating table.

Anesthesia was induced using ketamine at 2 mg/kg bwt and midazolam (Mezolam, VHB Medi Sciences Limited, Pantnagar, Uttarakhand, India) at 0.2 mg/kg bwt, i.v., administrated through the catheter. Induction was done in horses infused with preemptive ketamine immediately after recumbency and after 10 min of premedication in the control group. Quality of induction was scored as 1 (minimal or no muscle twitching, no movement, relaxed limbs), 2 (brief muscle rigidity followed by relaxation and minimal movement), 3 (marked muscle rigidity, movement, struggling), or 4 (fails to attain lateral recumbency) (18).

2.3. Maintenance of anesthesia

Anesthesia was maintained with i.v. ketamine at 2 mg/kg/h as a CRI. After induction, all horses were intubated and allowed to breathe spontaneously. An endotracheal tube was secured for administration of oxygen and to shift to inhalant anesthetic maintenance using volatile agents whenever necessary, particularly if the surgical procedure considerably exceeded more than 1 h. Bolus doses of i.v. thiopental (thiosol sodium) 5% solution were administered whenever an inadequacy in surgical anesthesia was observed.

Surgery was carried out according to the routine protocol of the clinic. Routine monitoring included HR, RR, RT, color of mucous membrane, gut movements, CRT, noninvasive mean arterial blood pressure (MABP), and peripheral oxygen saturation (SPO_2) (Globalvets 5000, Global Technology Solutions LLC, New Delhi, India), measured using appropriate instruments. Quality of surgical anesthesia was recorded as the average of the scores of degree of muscle relaxation and quality of analgesia and was scored by the same surgeon for all the horses, unaware of treatment allocation. Degree of muscle relaxation was scored as 1 (no trunk or limb twitching or movement, no resistance to flexion of limbs), 2 (slight trunk or limb muscle twitching, minimal resistance to flexion of limbs), 3 (strong trunk or limb muscle twitching, resistance to flexion of limbs), or 4 (muscle rigidity and strong resistance to flexion of limbs) (18). Quality of analgesia (i.e. response to surgical stimulation) was scored as 1 (no response to surgical stimulation), 2 (brief contraction of abdominal or limb muscles, temporary twitching or spasms), 3 (movement of a fore limb or hind limb), or 4 (repeated movement of a fore limb or hind limb requiring additional drug administration) (18). The total dose of thiopental used for maintenance of anesthesia (mg) and the duration of surgical procedure (min) were recorded. Local anesthetics and analgesics such as opioids, $alpha_2$ adrenergic receptor agonists, $beta_1$-adrenergic receptor agonists, steroids, and nonsteroidal antiinflammatory drugs (NSAIDs) were not used perioperatively or up to 2 h after surgery.

2.4. Postoperative period and recovery

After surgery, horses were allowed to recover in a padded recovery box under observation and manual assistance was given if necessary. Upon completion of surgery and discontinuation of anesthesia, the time to first head lift (min), time to sternal recumbency (min), and standing time (min) were recorded. The time to first head lift is the time from the discontinuation of anesthesia until first lifting of head. The time to sternal recumbency is the time from the discontinuation of anesthesia until the animal moves to sternal recumbency and the standing time is the time from the discontinuation of anesthesia until the animal is able to stand steadily. Quality of recovery from anesthesia was scored as 1 (unassisted, uneventful, 1 attempt to stand), 2 (unassisted, 2 or 3 attempts to stand), 3 (minimal assistance requiring 1 attendant because of >3 attempts to stand), or 4 (assistance to stand requiring 2 attendants to provide head and tail support because of moderate or severe ataxia) (18). Postoperative ataxia was scored as performed for scoring ataxia following the infusion of preemptive ketamine (4).

2.5. Hematological and serum biochemical observations

Routine monitoring of hematological and serological parameters, including hemoglobin (Hb), packed cell volume (PCV), differential leukocyte count (DLC), and total leukocyte count (TLC), was performed using appropriate equipment. Blood glucose estimations were done using a ROMOCHECK glucometer (Romsons Scientific & Surgical Pvt. Ltd., Nunhai, Agra, Uttar Pradesh, India). Serum biochemistry parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, cholesterol, and triglycerides were measured using appropriate diagnostic kits (Coral Clinical Systems, Bambolim Complex P. O., Goa, India) via spectrophotometric methods. Serum insulin, cortisol, and interleukin-6 (IL-6) were measured using horse-specific ELISA kits (BlueGene Biotech, Putuo, Shanghai, China).

Clinical, physiological, hematological, and serological parameters were monitored and recorded before drug administration (base value), at the point of anesthetic induction (0), and at 30, 60, 120, and 180 min after anesthetic induction.

2.6. zOverall pain score

The overall pain score was evaluated according to the composite pain score based on the parameters contributing to the behavior of the animal, response to observer, and physiological parameters (Table 1) (19). These parameters

Table 1. Multifactorial numerical rating composite pain scale used to score pain.

Parameters		Scores and description
Behavior of animal	Appearance	1. Bright, lowered head and ears, no reluctance to move 2. Bright and alert, occasional head movements, no reluctance to move 3. Restlessness, pricked up ears, abnormal facial expressions, dilated pupils 4. Excited, continuous body movements, abnormal facial expression
	Sweating	1. No obvious signs of sweat 2. Damp to the touch 3. Wet to the touch, beads of sweat apparent over the horse's body 4. Excessive sweating, beads of water running off the animal
	Kicking at abdomen	1. Quietly standing, no kicking 2. Occasional kicking at abdomen (1–2 times/5 min) 3. Frequent kicking at abdomen (3–4 times/5 min) 4. Excessive kicking at abdomen (>5 times/5 min), intermittent attempts to lie down and roll
	Pawing on floor	1. Quietly standing, no pawing 2. Occasional pawing (1–2 times/5 min) 3. Frequent pawing (3–4 times/5 min) 4. Excessive pawing (>5 times/5 min)
	Posture	1. Stands quietly, normal walk 2. Occasional weight shift, slight muscle tremors 3. Non-weight-bearing, abnormal weight distribution 4. Analgesic posture (attempts to urinate), prostration, muscle tremors
	Head movements	1. No evidence of discomfort, head straight ahead for the most part 2. Intermittent head movements laterally or vertically, looking at flanks (1–2/5 min), lip curling (1–2/5 min) 3. Intermittent and rapid head movements laterally or vertically, frequent looking at flanks (3–4/5 min), lip curling (3–4/5 min) 4. Continuous head movements, excessively looking at flanks (>5 times/5 min), lip curling (>5 times/5 min)
	Appetite	1. Eats hay readily or is not allowed to eat hay 2. Hesitates to eat hay 3. Shows little interest in hay, eats very little or takes hay into mouth but does not chew or swallow 4. Neither shows interest in nor eats hay
Response to observer	Interactive behavior	1. Pays attention to people 2. Exaggerated response to auditory stimulus 3. Excessive to aggressive response to auditory stimulus 4. Stupor, prostration, no response to auditory stimulus
	Response to palpation of the painful area	1. No reaction to palpation 2. Mild reaction to palpation 3. Resistance to palpation 4. Violent reaction to palpation

Table 1. (Continued).

Physiological data	Heart rate	1. 24–44 bpm 2. 45–52 bpm 3. 53–60 bpm 4. >60 bpm
	Respiratory rate	1. 8–13 breaths pm 2. 14–16 breaths pm 3. 17–18 breaths pm 4. >18 breaths pm
	Rectal temperature	1. 36.98–38.58 °C 2. 36.48–36.98 °C or 38.58–39.08 °C 3. 35.98–36.48 °C or 39.08–39.58 °C 4. 35.48–35.98 °C or 39.58–40.08 °C
	Digestive sounds (bowel movements)	1. Normal motility 2. Decreased motility 3. No motility 4. Hypermotility

were recorded in all horses before any drugs were given (base value), at 120 min (1 h after the end of surgery), and at 180 min (2 h after the end of surgery). The cumulative score was obtained by adding individual observations with a range of 13–52. Additional analgesics, NSAIDs, or other treatments were given 2 h after the end of surgery as deemed appropriate by the clinician. Any adverse events were also documented.

2.7. Data analysis

All statistical analysis was performed using the statistical software package SAS 9.3. The data from horses in the LK and HK groups were compared with those in group S. The primary outcome of the study was to measure the quality of sedation, down-time, quality of analgesia, and quality of anesthetic induction with a null hypothesis that there would be no differences between the groups. Secondary outcome measures were physiological variables, quality of recovery, and degree of postoperative pain and ataxia with a null hypothesis of no differences between the groups. Single measurements of continuous variables between groups were compared using one-way analysis of variance (ANOVA). Continuous data of parametric variables within and between groups collected at different time intervals were analyzed using repeated measures ANOVA. For both one-way ANOVA and repeated measures ANOVA, the post hoc Tukey HSD test was employed. Nonnormally distributed data or nonparametric variables (scores) at different intervals were compared using the Kruskal–Wallis test. Dunn's test was used for analyzing pairwise differences in groups if there was a tied rank for the Kruskal–Wallis test (20).

3. Results

3.1. Horse details

Eighteen horses admitted for short- to medium-term surgical procedures were randomly divided into three groups (Table 2). There was no significant difference between groups with regard to body weight or age ($P > 0.05$).

3.2. Preemptive ketamine infusion, premedication, and induction

During and after preemptive ketamine infusion, frequent shifting of weight in limbs was evident in ketamine-infused horses and its frequency was slightly higher in group HK. Ataxia was found to be significantly higher ($P < 0.05$) in group HK than in group S (Table 3) during the lag period. The mucous membrane was pale pink in all horses. HR, RR, and RT did not show much deviation from base values during i.v. ketamine infusion or during the lag period (Figure 1).

All horses were visibly sedated after the preanesthetics were given immediately prior to induction. Clinically, sedation was better among horses that received subanesthetic doses of the preemptive ketamine infusions compared to group S, with the most significant ($P < 0.05$) sedation scores in the HK group (Table 3). The mean down-time in preemptive ketamine-infused horses in groups HK and LK were 2 ± 0.32 min and 5 ± 0.32 min, respectively. A dose-dependent significantly shorter down-time ($P < 0.05$) was noticed between groups LK and HK. Anesthesia was induced with ketamine and midazolam once the horse was recumbent. The median induction score was excellent in the preemptive ketamine-infused group (Table 3).

Table 2. Horse details, surgical condition, grouping of animals into three groups. S_{1-6}: Saline, LK_{1-6}: low ketamine, and HK_{1-6}: high ketamine, n = 6 in each group.

Groups	Age (years)	Body weight (Kg)	Sex	Surgical condition
S_1	5	243	M	Laceration
S_2	7	300	F	Barbed wire injury
S_3	8	178	F	Barbed wire injury
S_4	8	430	M	Castration
S_5	7	303	F	Metacarpal fracture
S_6	6	250	M	Barbed wire injury
Mean ± SE	6.83 ± 0.48	284 ± 34.64	-	-
LK_1	4	180	M	Tumor at scapular region
LK_2	6	220	M	Barbed wire injury
LK_3	7	295	M	Barbed wire injury
LK_4	3	242	F	Tongue laceration
LK_5	7	276	F	Tongue laceration
LK_6	6	280	F	Barbed wire injury
Mean ± SE	5.5 ± 0.67	248.83 ± 17.76	-	-
HK_1	3	280	M	Castration
HK_2	6	193	M	Eyelid growth
HK_3	3	242	F	Tongue laceration
HK_4	7	383	F	Barbed wire injury
HK_5	10	215	M	Extirpation of eye
HK_6	6	275	M	Barbed wire injury
Mean ± SE	5.83 ± 1.08	264.67 ± 27.36	-	-

Table 3. Median ± SD of nonparametric scores and mean ± SE of down-time, thiopentone used to maintain anesthesia, duration of surgery, first head lift, sternal recumbency, and standing time in groups S, LK, and HK. ^{a,b,c} Values with different superscript letters differ significantly ($P < 0.05$) in respective parameters.

Variable	S (normal saline CRI alone for 30 min), n = 6	LK (preemptive ketamine @ 0.2 mg/kg CRI for 30 min), n = 6	HK (preemptive ketamine @ 0.4 mg/kg CRI for 30 min), n = 6
Ataxia after preemptive ketamine	1 ± 0^{a}	2 ± 0^{ab}	3 ± 0.41^{b}
Quality of sedation	2.5 ± 1.21^{a}	1 ± 0.84^{ab}	1 ± 0^{b}
Quality of induction	1.5 ± 0.55	1 ± 0	1 ± 0.41
Quality of muscle relaxation	1 ± 0.52	1 ± 0	1 ± 0
Quality of analgesia	1 ± 0	1 ± 0	1 ± 0
Post anesthetic ataxia	2 ± 0.89^{a}	3.5 ± 0.55^{b}	4 ± 0.52^{b}
Quality of recovery	2 ± 0.41	2 ± 0.75	2 ± 0.41
Down-time (min)	-	4.83 ± 0.31^{b}	2 ± 0.26^{c}
Thiopentone dose (mg)	2500 ± 718.8	1750 ± 359.4	1541.67 ± 305.62
Duration of surgery (min)	77 ± 13.78	73.67 ± 9.92	49.5 ± 6.96
Time to first head lift (min)	29.83 ± 5.13	27.67 ± 3.54	23.67 ± 5.81
Time to sternal recumbency(min)	61 ± 11.53	52.5 ± 7.27	39 ± 5.6
Standing time (min)	76.33 ± 12.09	64 ± 9.29	44.17 ± 3.45

However, there was no significant difference (P > 0.05) in the quality of induction between the groups. Although a significant difference in quality of induction was not observed, 11 out of 12 preemptive ketamine-infused horses attained smooth induction compared to horses anesthetized without preemptive ketamine (3/6). HR and RR decreased significantly (P < 0.05) during induction in all three groups (Figure 1) without any intergroup differences (P > 0.05). There was no significant difference (P > 0.05) in MABP during induction between groups.

3.3. Maintenance of anesthesia

Anesthesia was maintained with i.v. CRIs of ketamine and thiopental bolus alone. All the horses received a perioperative crystalloid solution. No additional drugs and/or volatile agents were used for maintenance of anesthesia. Mucous membrane was pale pink in all horses. There was no significant difference (P > 0.05) in values of HR, RR, MABP, and SPO_2 between groups. The fluctuations in HR, RR, MABP, and SPO_2 during maintenance of anesthesia were less evident in group HK compared to groups S and LK (Figures 1 and 2). Quality of muscle relaxation (scores 1 and 2) and quality of analgesia (score 1) were excellent in all groups during the perioperative period without any significant differences (P > 0.05). Preemptive infusion of ketamine prior to premedication did not significantly affect any results related to maintenance of anesthesia (P > 0.05). There was no significant difference (P > 0.05) in the amount of thiopental (mg) used to maintain anesthesia between groups. Also, there was no significant difference (P > 0.05) in the duration of surgery between groups (Table 3). RT decreased significantly (P < 0.05) at 60 min in all the groups (Figure 1).

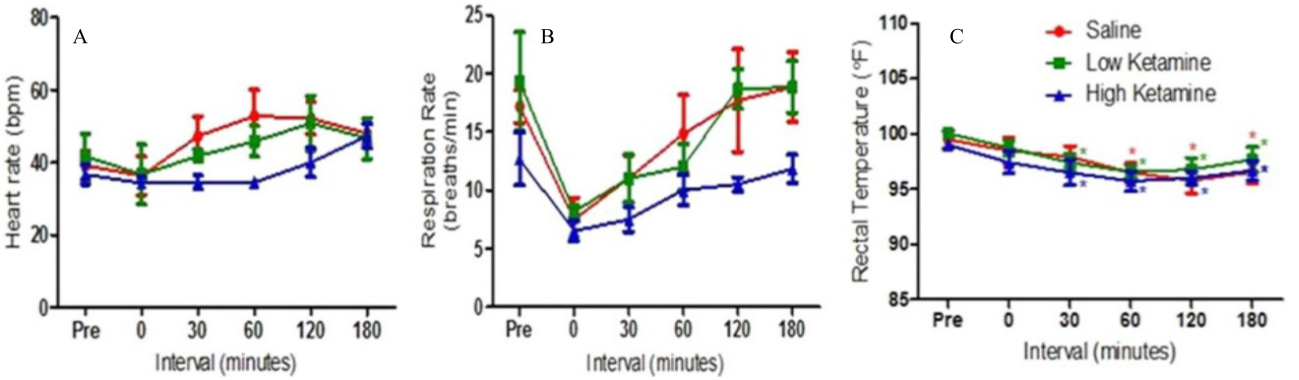

Figure 1. Mean ± SE values of heart rate (A), respiration rate (B), and rectal temperature (RT) in groups S (normal saline CRI), LK (ketamine at 0.2 mg/kg bwt CRI), and HK (ketamine at 0.4 mg/kg bwt CRI) at different intervals [pre (before administration of any drug), 0 (induction), 30, 60, 120, and 180 min], n = 6.

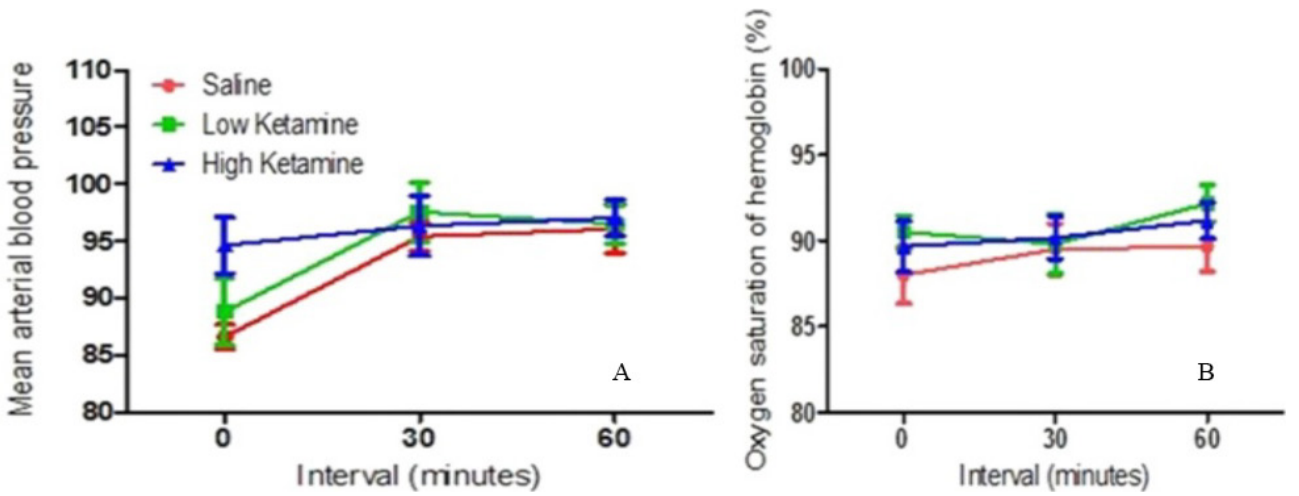

Figure 2. Mean ± SE values of mean arterial blood pressure (A) and oxygen saturation of hemoglobin (B) in groups S (normal saline CRI), LK (ketamine at 0.2 mg/kg bwt CRI), and HK (ketamine at 0.4 mg/kg bwt CRI) at different intervals [0 (induction), 30, and 60 min], n = 6.

3.4. Postoperative period and recovery

There was no significant difference ($P > 0.05$) in the time taken for first head lift, time to sternal recumbency, standing time, or quality of recovery between groups. The time taken for the first head lift was nearly 30 min for all horses. The time to sternal recumbency and standing time in group S took longer, followed by groups LK and HK. All the horses stood in less than 2 h after the end of surgery with noticeably good recovery scores of 2 ± 0.41, 2 ± 0.75, and 2 ± 0.41 in groups S, LK, and HK, respectively. In group HK, all the horses stood in less than 1 h, although postanesthetic ataxia was significantly higher ($P < 0.05$) compared to group S (Table 3). Dose-dependent significance ($P > 0.05$) in postanesthetic ataxia was also not observed. HR and RR remained within normal limits in all groups during the postoperative period (Figure 1). There was a significant ($P < 0.05$) reduction in RT from base values 1 h after the end of surgery in all groups (Figure 1).

3.5. Hematological and serum biochemical observations

There was no specific pattern of change or clinical significance in hematological and serum biochemical parameters in all three groups throughout the observation period except for values of glucose (Table 4). Glucose increased clinically in all three groups throughout the perioperative period and was significantly higher ($P < 0.05$) from the base values in group S at 30 and 60 min and also from the other two groups (Table 4; Figure 3). The insulin, cortisol, and IL-6 values fluctuated near the base values in all groups throughout the study (Table 4). The base values of IL-6 in group S were significantly ($P < 0.05$) higher among and between groups.

3.6. Overall pain score

The overall pain score increased clinically at 120 min after induction from the base value and decreased towards base values at 180 min in all groups (Figure 4). Although statistically insignificant ($P > 0.05$), the fluctuation in postoperative pain scores at 1 h and 2 h after the end of surgery was minimal in the HK group. All the horses were clinically and physiologically stable without any sedation or ataxia before 2 h after surgery. Additional NSAIDs were given to all horses after 2 h postoperatively.

3.7. Adverse effects

No animals had any adverse effects or suffered any significant perioperative or postoperative morbidity or mortality. Mild box walking was noticed in a few horses of all three groups, which was controlled by normal head restraint.

4. Discussion

The aim of this study was to investigate the effects of preemptive ketamine infusion given prior to premedication in horses subjected to a conventional anesthetic protocol. The hypotheses that preemptive ketamine infusion would enhance sedation and reduce down-time were supported. Although statistically insignificant, clinically, preemptive ketamine infusion in horses at 0.4 mg/kg bwt enabled smooth anesthetic induction, anesthetic maintenance with minimum fluctuations in physiological variables and vital functions, and reduced postoperative pain.

The horses were randomly divided into groups irrespective of body weight, age, and the procedure for which they were admitted to the clinic. The drugs used for anesthesia, other than the test drug, were kept the same for all horses in order to maintain uniformity among groups, reduce bias, and retain normal clinical settings. Ketamine was used as the sole induction agent along with midazolam, representing its recent wide acceptance for this purpose in horses (21,22). To minimize the use of additional drugs for maintenance of anesthesia and thereby reduce the possible variations and interactions on the effect of the test drug, a TIVA protocol was chosen. TIVA is less stressful to horses and has fewer perioperative fatalities than anesthetic maintenance with volatile agents for surgical procedures of less than 90 min in duration (23). Isoflurane is routinely used in our clinics for maintenance of anesthesia, but we used ketamine CRI at 2 mg/kg/h along with bolus doses of thiopental whenever necessary. The CRI of i.v. ketamine at 2 mg/kg/h is routinely used as a maintenance anesthetic in properly sedated and anesthetized horses as it retains stable cardiovascular function (3,4). In order to minimize inhalant anesthetic requirements and the potential hazards such as hypotension and respiratory depression associated with the use of inhalant anesthetics, it is often necessary to administer additional drugs and supplementary anesthetics (22). For the above reasons, and considering the short to medium duration of the surgical procedures performed, volatile agents were not used for maintenance of anesthesia. The i.v. administration of thiopental to properly premedicated normal healthy horse produces only mild hemodynamic changes (24). Moreover, the hazards associated with bolus dosing of thiopental were not encountered in any of the horses when maintained along with CRI of ketamine. The cardiopulmonary and physiological variables reflected the expected outcome during maintenance of anesthesia, shown by no significant differences between variables in all the groups and minimum fluctuation in the high preemptive ketamine-infused group. It is recognized that CRI of ketamine has no substantial effects on the respiratory system (8). Significant bradycardia and decreased MABP were detected 6 h after ketamine CRI in a previous study (8). The CRI of ketamine used in our study for short- to medium-term infusion did not show any such significant cardiovascular depressing effects. Whenever a surgical procedure exceeded or tended to exceed considerably more than 60–90 min, the horses were shifted to inhalant anesthesia and were excluded from the study.

Table 4. Mean ± SE of glucose, insulin, cortisol, and IL-6 values in groups S (normal saline CRI), LK (ketamine at 0.2 mg/kg bwt CRI), and HK (ketamine at 0.4 mg/kg bwt CRI for 30 min and 30 min before preanesthetics were given), n = 6. $^{a,b,c,\alpha,\beta,\gamma}$ Values with different superscript letters differ significantly ($P < 0.05$) in corresponding intervals in respective parameters.

Variables	Groups	Interval (min)					
		Base value	0	30	60	120	180
Glucose (mg/dL)	S	153.83 ± 29.07^{bcd}	154.83 ± 34.77^{bcd}	227.83 ± 35.73$^{a\alpha}$	222 ± 34.75$^{a\alpha}$	193.83 ± 27.95^{abc}	205.67 ± 24.99^{ab}
	LK	131.17 ± 17.36^{d}	140.2 ± 16.85^{cd}	155.53 ± 23.37$^{bcd\beta}$	146.83 ± 23.83$^{cd\beta}$	149.5 ± 22.56^{cd}	145.93 ± 14.22^{cd}
	HK	112.67 ± 11.95^{d}	114.17 ± 10.24^{d}	153 ± 15.04$^{bcd\gamma}$	144.5 ± 13.24$^{cd\gamma}$	153.67 ± 10.17^{bcd}	149.33 ± 16.62^{cd}
Insulin (pg/dL)	S	7.47 ± 0.98^{a}	7.06 ± 0.66^{ab}	6.4 ± 1.03^{abcd}	6.4 ± 0.85^{abcd}	6.45 ± 0.96^{abcd}	6.67 ± 1.06^{abc}
	LK	3.43 ± 1.33^{f}	3.61 ± 1.26^{f}	3.14 ± 1.28^{f}	3.86 ± 1.^{25ef}	3.6 ± 1.43^{f}	3.64 ± 1.38^{f}
	HK	5.35 ± 1.53^{cd}	5.11 ± 1.47^{de}	5.31 ± 1.48^{d}	5.77 ± 1.71^{bcd}	5.68 ± 1.67^{cd}	5.11 ± 1.92^{de}
Cortisol (ng/dL)	S	56.9 ± 11.2^{a}	49.38 ± 9.7^{a}	47.25 ± 11.04^{a}	47 ± 8.62^{a}	45.12 ± 8.63^{a}	54.54 ± 9.29^{a}
	LK	46.22 ± 32.46^{a}	71.87 ± 56.72^{a}	94.52 ± 81.64^{a}	27.82 ± 13.2^{a}	34.02 ± 18.87^{a}	62.4 ± 46.18^{a}
	HK	48.79 ± 25.83^{a}	48.85 ± 25.88^{a}	45.84 ± 26.22^{a}	36.84 ± 21.45^{a}	31.34 ± 17.51^{a}	31.63 ± 18.02^{a}
IL-6 (pg/dL)	S	373.54 ± 129.37^{a}	199.52 ± 30.2^{b}	239.76 ± 38.39^{ab}	224.68 ± 47.29^{ab}	224.6 ± 52.94^{ab}	145.37 ± 37.23^{b}
	LK	110.27 ± 28.59^{b}	119.65 ± 32.23^{b}	125.54 ± 36.08^{b}	115.35 ± 19.69^{b}	127.41 ± 26.69^{b}	138.21 ± 32.74^{b}
	HK	176.45 ± 52.64^{b}	159.3 ± 47.37^{b}	150.56 ± 43.77^{b}	159.85 ± 49.51^{b}	133.38 ± 30.41^{b}	144.71 ± 28.47^{b}

A wide range of surgical procedures performed in equine clinical practice were included in this study. The pain inflicted by these procedures may vary from mild to severe. Quality of analgesia during the procedure was scored as excellent in all the horses because of the adequacy of pain control. Although contradictory reports on analgesic effects of ketamine at subanesthetic doses in normal awake horses exist (8), in horses suffering from pain, inflammatory tissue injury, and/or endotoxemia, ketamine at subanesthetic doses has been documented to possess analgesic and antiinflammatory activity (11). Our deliberation was on whether infusion of a subanesthetic dose of ketamine as a preemptive analgesic before the initial surgical stimulus prevented the establishment of an altered processing of pain stimuli during surgery and in the immediate postoperative period. Postoperative pain scores were excellent in all horses, irrespective of group, 2 h after surgery. The hypothesized analgesic effect of preemptive ketamine infusion in reducing postoperative pain could not be evaluated meticulously because of possible cumulative interference of the effects of preoperative butorphanol, xylazine, and ketamine and perioperative CRI of ketamine. Evidence suggests that butorphanol may be efficacious for treatment of visceral pain in horses and possesses minimum effects in sharp superficial and deep somatic pain (25,26). Preemptive and/or perioperative ketamine infusion is thought to have mitigated the perioperative and postoperative superficial and deep somatic pain caused by surgical procedures. The adverse effects were also less, unlike opioids with anticipated reduction in propulsive gastrointestinal motility (27).

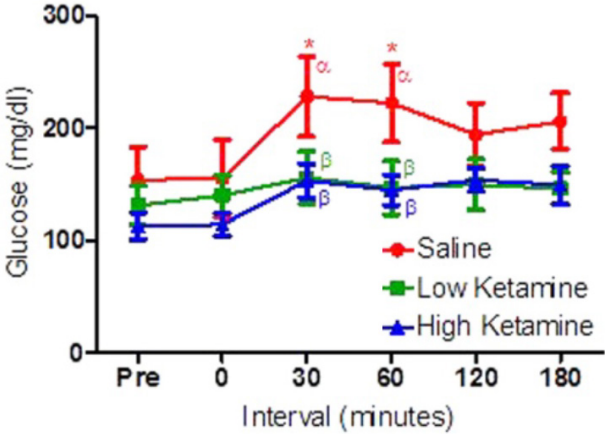

Figure 3. Mean ± SE values of glucose in groups S (normal saline CRI), LK (ketamine at 0.2 mg/kg bwt CRI), and HK (ketamine at 0.4 mg/kg bwt CRI) at different intervals [pre (before administration of any drug), 0 (induction), 30, 60, 120, and 180 min], n = 6, *: significant ($P < 0.05$) change from pre value in group S; $^{*,\alpha,\beta}$: significant difference ($P < 0.05$) between groups at corresponding intervals.

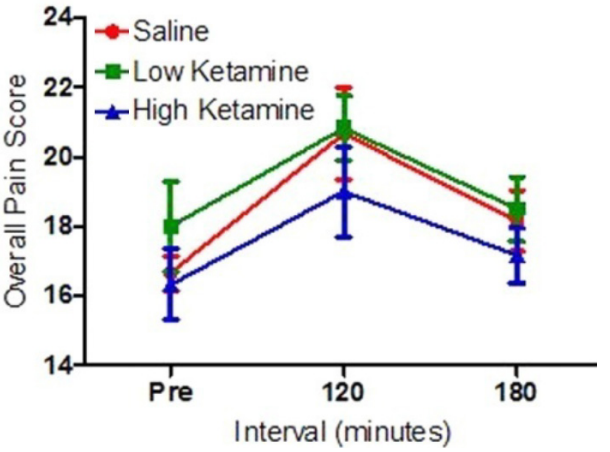

Figure 4. Mean ± SE values of overall pain score in groups S (normal saline CRI), LK (ketamine at 0.2 mg/kg bwt CRI), and HK (ketamine at 0.4 mg/kg bwt CRI) at different intervals [pre (before administration of any drug), 1 h after the end of surgery, and 2 h after the end of surgery], n = 6.

This study was also intended to compare the effects of preemptive ketamine infusion at two different doses, 0.2 mg/kg bwt and 0.4 mg/kg bwt, in horses usually premedicated with xylazine and butorphanol. The dose rates were selected based on results from previous studies (8,9). CRI of ketamine at dose rates from 0.4 mg/kg/h to 1.5 mg/kg/h has been safely administered for prolonged duration in healthy awake horses without any adverse effects while higher doses, above 1.5 mg/kg/h, for a longer period showed excitation (8). A delayed gastrointestinal transit time was reported in horses with infusions at 1.2 mg/kg/h for more than 24 h (28). Lower doses of ketamine were selected for preemptive infusion in order to avoid any disturbing effects of NMDA receptor antagonism like excitation, tremors, and ataxia. Preemptive ketamine-infused horses achieved recumbency before any induction agents were given. This has been referred to as down-time in this study. A dose-dependent shorter down-time was observed in preemptive ketamine-infused horses. At higher doses of 0.4 mg/kg bwt, preemptive ketamine further reduced down-time and the horses were adequately sedated. Subanesthetic doses of preemptive ketamine infusions in normal awake horses lack sedative effects, but they effectively augment sedation at higher dose rates of infusion when combined with xylazine and butorphanol (6). Initiation of preanesthetic drugs 30 min after ketamine infusion complemented the action of the preanesthetic drugs. Heart rate decreased during induction of anesthesia in all the horses, attributed to baroreceptor activation by the alpha$_2$ adrenoceptor agonist, but the variation in different perioperative intervals was minimal in horses infused with a high dose of preemptive ketamine. High-dose ketamine infusion preserved the sympathetic drive and minimized the fluctuations in cardiorespiratory parameters caused by xylazine compared to low preemptive ketamine infusion. Ketamine induction and perioperative ketamine CRI appeared to obliterate any potential dose-dependent anesthetic sparing effects of preemptive ketamine infusion. Shifting of weight and dose-dependent ataxia was evident during the lag period in horses infused with preemptive ketamine at 0.4 mg/kg bwt. Upon standing postoperatively, the preemptive ketamine-infused horses were significantly more ataxic.

The fluctuation in postoperative pain scores at 1 h and 2 h after the end of surgery was considerably less in horses infused with preemptive ketamine at 0.4 mg/kg bwt. Owing to the small number of horses in each group and the small difference in the range of doses of preemptive ketamine chosen for evaluation, it was difficult to reach a dose-dependent conclusive result on postoperative pain. Further investigation is hence warranted at higher dose rates as preemptive infusion. As CRI of ketamine was given to all horses perioperatively, ataxia and decline in rise of postoperative pain may have been expected to occur in almost equal ranges of scores among all three groups. However, insignificant variations were noticed between groups. A triggering effect of preemptive ketamine in inhibiting altered pain processing or an additive effect along with perioperative ketamine is hence thought to have occurred in the horses. A better understanding of the dynamic action of preemptive ketamine may be obtained if the plasma drug concentration is measured and compared with clinical effects. An initial distributive phase of nearly

2.3 min followed by slow elimination with a half-life of 67.4 min has been estimated for CRI of ketamine at 1.5 mg/kg/h in healthy awake horses (9). During infusion of ketamine at 0.4 mg/kg/h for 6 h in a study, the half-life of disappearance estimated was 2.16 h (8). The clearance and half-life of ketamine are faster in normal awake animals than those sedated and anesthetized (29,30). The elimination of ketamine from circulation is hence thought to have been decreased by the low infusion rates of preemptive ketamine, and it may be further delayed by sedatives and anesthetics. The perioperative CRI of ketamine might have compensated the likely fall in plasma concentration of preemptively infused ketamine over time. This might have been reflected in the perioperative and immediate postoperative effects in horses infused with preemptive ketamine. We did not measure the ketamine in the blood following preemptive infusion or postoperatively, but shifting of weight and ataxia in preemptive ketamine-infused horses alone substantiated the effect of ketamine.

The hematological and serum biochemical parameters invariably remained the same throughout the study in all groups. In horses anesthetized without preemptive ketamine infusion, a significantly high rise in glucose during the perioperative period was observed. Alpha$_2$ adrenoceptor agonists induce hyperglycemia by inhibiting insulin release (31), but the profound hyperglycemia noticed only in horses anesthetized without preemptive ketamine showed an apparent surgical stress-induced hyperglycemia due to gluconeogenesis. Infusion of preemptive ketamine did not show any effect on serum insulin and cortisol levels. In normal awake horses, ketamine increases catecholamines and raises the circulating plasma cortisol because of its sympathomimetic effects (9). Rather than as CRI alone, ketamine when used along with sedatives and anesthetics has been shown to delay the rise in cortisol level and mitigate the stress response (10). The preoperative and/or perioperative CRI of ketamine is hence thought to have facilitated amelioration of the stress response to surgery. The high base value of IL-6, a major factor in acute phase immune responses, in group S may correspond to the inherent inflammatory changes due to the surgical condition of horses in this group. The members of the IL-6 family have proinflammatory effects in addition to antiinflammatory effects such as downregulation of the inflammatory cascade by inhibiting TNF-alpha synthesis (32). The preemptive and/or perioperative infusion of ketamine maintained IL-6 base values throughout the study. Further investigation may be necessary from a broad perspective to assess their roles in contending with inflammation during ketamine administration.

No adverse effects or postoperative morbidity were observed in any of the horses enrolled in the study except for mild box walking, which is consistently reported in horses premedicated with butorphanol (22). The locomotor activity after elective surgeries under i.v. anesthesia was noticed in ponies premedicated with opioids (33).

In conclusion, preemptive ketamine infusion prior to premedication in horses at 0.4 mg/kg bwt enhances quality of sedation and shortens the down-time. Although statistical significance was not observed, clinically, preemptive ketamine infusion in horses at 0.4 mg/kg bwt enabled smooth anesthetic induction with minimum fluctuations in perioperative physiological and vital functions and reduced postoperative pain. More clinical studies to investigate the effects of subanesthetic doses of preemptive ketamine in a larger population may be warranted prior to routine use in anesthetic practice.

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Author Contributions

Pallvi Sharma wrote the manuscript. Aswathy Gopinathan and Kiranjeet Singh contributed to the conception and design of the study. Sherin B. Sarangom performed critical revisions of data analysis and the manuscript. Chelladurai Sowbharenya and Christina John assisted in all the anesthetic and surgical procedures. Med Ram Verma contributed to the data analysis. All authors made substantial contributions to acquisition, analysis, and interpretation of data in addition to laboratory analysis, surgical interventions, further revision of the manuscript, and its final approval.

References

1. Visser E, Schug SA. The role of ketamine in pain management. Biomed Pharmacother 2006; 60: 341-348.

2. Sarton E, Teppema LJ, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL, Dahan A. **The involvement of the mu-opioid receptor in ketamine-induced respiratory depression and antinociception.** Anesth Analg 2001; 93: 1495-1500.

3. McMurphy RM, Young LE, Marlin DJ, Walsh K. Comparison of the cardiopulmonary effects of anesthesia maintained by continuous infusion of romifidine, guaifenesin, and ketamine with anesthesia maintained by inhalation of halothane in horses. Am J Vet Res 2002; 63: 1655-1661.

4. Mama KR, Wagner AE, Steffey EP , Kollias-Baker C, Hellyer PW , Golden AE, Brevard LF. Evaluation of xylazine and ketamine for total intravenous anesthesia in horses. Am J Vet Res 2005; 66: 1002-1007.

5. Peterbauer C, Larenza PM, Knobloch M, Theurillat R, Thormann W, Mevissen M, Spadavecchia C. Effects of a low dose infusion of racemic and S-ketamine on the nociceptive withdrawal reflex in standing ponies. Vet Anaesth Analg 2008; 35: 414-423.

6. Wagner AE, Mama KR, Contino EK, Ferris DJ, Kawcak CE. Evaluation of sedation and analgesia in standing horses after administration of xylazine, butorphanol, and subanesthetic doses of ketamine. J Am Vet Med Assoc 2011; 238: 1629-1633.

7. Guedes AG, Matthews NS, Hood DM. Effect of ketamine hydrochloride on the analgesic effects of tramadol hydrochloride in horses with signs of chronic laminitis-associated pain. Am J Vet Res 2012; 73: 610-619.

8. Fielding CL, Brumbaugh G, Matthews NS, Peck KE, Roussel AJ. Pharmacokinetics and clinical effects of a subanesthetic continuous rate infusion of ketamine in awake horses. Am J Vet Res 2006; 67: 1484-1690.

9. Lankveld DPK, Driessen B, Soma LR, Moate PJ, Rudy J, Uboh CE, Van Dijk P, Hellebrekers LJ. Pharmacodynamic effects and pharmacokinetic profile of a long-term continuous rate infusion of racemic ketamine in healthy conscious horses. J Vet Pharmacol Ther 2006; 29: 477-488.

10. Taylor PM. Equine stress responses to anaesthesia. Br J Anaesth 1989; 63: 702-709.

11. Lankveld DPK, Bull S, Van Dijk P, Fink-Gremmels J, Hellebrekers LJ. Ketamine inhibits LPS-induced tumour necrosis factor-alpha and interleukin-6 in an equine macrophage cell line. Vet Res 2005; 36: 257-262.

12. Kushiro T, Yamashita K, Umar MA, Maehara S, Wakaiki S, Abe R, Seno T, Tsuzuki K, Izumisawa Y, Muir WW. Anesthetic and cardiovascular effects of balanced anesthesia using constant rate infusion of midazolam-ketamine- medetomidine with inhalation of oxygen-sevoflurane (MKM-OS anesthesia) in horses. J Vet Med Sci 2005; 67: 379-384.

13. Young LE, Bartram DH, Diamond MJ, Gregg AS, Jones RS. Clinical evaluation of an infusion of xylazine, guaifenesin and ketamine for maintenance of anaesthesia in horses. Equine Vet J 1993; 25: 115-119.

14. Muir WW 3rd, Gadawski JE, Grosenbaugh DA. Cardiorespiratory effects of a tiletamine/zolazepam-ketamine-detomidine combination in horses. Am J Vet Res 1999; 60: 770-774.

15. Karine P, Keila KI. The ASA physical status classification: What is the evidence for recommending its use in veterinary anesthesia?—A systematic review. Front Vet Sci 2018; 5: 204.

16. Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. Equine Vet J 1988; 20: 41-45.

17. Keates HL, Van Eps AW, Pearson MR. Alfaxalone compared with ketamine for induction of anaesthesia in horses following xylazine and guaifenesin. Vet Anaesth Analg 2012; 39: 591-598.

18. Muir WW 3rd, Lerche P, Robertson JT, Hubbell JA, Beard W, Miller T, Badgley B, Bothwell V. Comparison of four drug combinations for total intravenous anesthesia of horses undergoing surgical removal of an abdominal testis. J Am Vet Med Assoc 2000; 217: 869-873.

19. Bussieres G, Jacques C, Lainay O, Beauchamp G, Leblond A, Cadore JL, Desmaizieres LM, Cuvelliez SG, Troncy E. Development of a composite orthopaedic pain scale in horses. Res Vet Sc 2008; 85: 294-306.

20. Elliott AC, Hynan LS. A SAS (*) macro implementation of a multiple comparison post hoc test for a Kruskal-Wallis analysis. Comput Methods Programs Biomed 2011; 102: 75-80.

21. De Vries A, Thomson S, Taylor PM. Comparison of midazolam and diazepam as co-induction agents with ketamine for anaesthesia in sedated ponies undergoing field castration. Vet Anaesth Analg 2015; 42: 512-517.

22. Taylor PM, Hoare HR, De Vries A, Love EJ, Coumbe KM, White KL, Murrell JC. A multicentre, prospective, randomised, blinded clinical trial to compare some perioperative effects of buprenorphine or butorphanol premedication before equine elective general anaesthesia and surgery. Equine Vet J 2016; 48: 442-450.

23. Johnston GM, Eastment JK, Wood J, Taylor PM. The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of phases 1 and 2. Vet Anaesth Analg 2002; 29: 159-170.

24. Muir WW 3rd, Mason DE. Effects of diazepam, acepromazine, detomidine, and xylazine on thiamylal anesthesia in horses. J Am Vet Med Assoc 1993; 203: 1031-1038.

25. Kalpravidh M, Lumb WV Wright M, Heath RB. Effects of butorphanol, flunixin, levorphanol, morphine, and xylazine in ponies. Am J Vet Res 1984; 45: 217-223.

26. Spadavecchia C, Arendt-Nielsen L, Spadavecchia L, Mosing M, Auer U, Van Den Hoven R. Effects of butorphanol on the withdrawal reflex using threshold, suprathreshold, and repeated subthreshold electrical stimuli in conscious horses. Vet Anaesth Analg 2007; 34: 48-58.

27. Boscan P, Van Hoogmoed LM, Farver TB, Snyder JR. Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. Am J Vet Res 2006; 67: 992-997.

28. Elfenbein JR, Robertson SA, Corser AA, Urion RJ, Sanchez LC. Systemic effects of a prolonged continuous infusion of ketamine in healthy horses. J Vet Intern Med 2011; 25: 1134-1137.

29. Waterman AE. The pharmacokinetics of ketamine administered intravenously in calves and the modifying effect of premedication with xylazine hydrochloride. J Vet Pharmacol Ther 1984; 7: 125-130.

30. Waterman AE, Robertson SA, Lane JG. Pharmacokinetics of intravenously administered ketamine in the horse. Res Vet Sci 1987; 42: 162-166.

31. Thurmon JC, Steffey EP, Zinkl JG, Woliner M, Howland D Jr. Xylazine causes transient dose-related hyperglycemia and increased urine volumes in mares. Am J Vet Res 1984; 45: 224-227.

32. Epstein A, Nir E, Eyngor M, Eldar A, Bdolah-Abram T, Kelmer E, Steinman A, Bruchim Y. Dynamic of cytokine gene transcription (TNF-α, IL-1β, IL-6, IL-8) in surgically treated colic horses by use of real-time PCR (RT-PCR). Isr J Vet Med 2016; 71: 24-30.

33. Rigotti C, De Vries A, Taylor PM. Buprenorphine provides better anaesthetic conditions than butorphanol for field castration in ponies: results of a randomised clinical trial. Vet Rec 2014; 175: 623.