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Anaesthetic and cardiopulmonary evaluation following xylazine-Diazepam-ketamine-propofol administration with or without local infiltration analgesia using mepivacaine during inguinal castration in Donkeys

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ABSTRACT

The present study was performed to investigate the cardiopulmonary responses and hypoalgesic efficacy of diazepam/ketamine anaesthesia induction followed by xylazine-propofol infusion alone (XDKP) or with a local infiltration of mepivacaine (XDKPM) for inguinal castration in donkeys. A 10 male adult donkeys were assigned to two groups (XDKP and XDKPM). Donkeys were sedated by xylazine then anaesthesia was inducted with followed by diazepam/ketamine. Anaesthesia was maintained by propofol infusion 0.1 mg/kg/h. XDKPM group, mepivacaine 2% were locally infiltrated on each side and two min before surgical incision. Both group got smooth induction and recovery. The pain scale scores showed a significant higher compared to baseline in both groups. In conclusion, a combination of XDKP is balanced total intravenous anaesthesia regimen with cardiopulmonary stability in donkeys undergoing inguinal castration.

1. INTRODUCTION

Donkeys are exposed to the different surgical interventions which elicited painful conditions such as musculoskeletal disorders, colic and wounds (Regan et al., 2016). Currently, the identification and detection of the specific pain behaviors in the donkey are not fully understood. The effectiveness of analgesics is measured by the response of animals and changes of the pain behavioral response (Ashley et al., 2005, Regan et al., 2016). The effectiveness of analgesia is measured by response of animals and changes of the behavioral response (Friton et al., 2006).

There were a few studies that concerned with the pharmacokinetic or pharmacodynamic of the analgesics during visceral or somatic pain in donkeys, unlike horses. The specific pain behaviors in working donkey were analyzed in recent study to improve the animal's welfare and management of pain (Regan et al., 2016).

Pain management in the donkey depends on using of multi-model analgesic to inhibit the inflammatory cascade. It is challenging to find the optimal field anesthetic regimen in a donkey which is able to overcome the fatality rate and produce a balanced anesthetic combination with profound analgesia (Mason et al., 2005).

Alpha 2 agonist are sedative and hypoalgesic drugs in equine such as xylazine which has a short analgesic duration (15–20) min in donkeys; and its dose is similar to horses (0.6 up to 1.1 mg/kg) (Lizarraga and Beths, 2012).

Many anaesthetic combinations had been used in donkeys, to name a few; the intermittent bolus of xylazine/ketamine every 10 min or a triple drip combination of xylazine, ketamine and guaifenesin. (Matthews et al., 2002, Matthews and van Loon, 2013). Added diazepam to ketamine during anaesthesia induction in donkeys to decrease the risk of cardiopulmonary depression and prolong the anaesthesia duration with smooth recovery (Matthews et al., 2005, Abakar et al., 2014).

Propofol has a longer induction time and higher metabolism compared to thiopental so that it has either no accumulative or apneic effect besides, it produces a smooth recovery (Abd-Almaseeh, 2008). Using a sedative dose of xylazine followed by ketamine/ propofol produced better induction and good muscle relaxation with longer duration of anesthesia and smooth recovery in donkeys than a

used ketamine or propofol alone. The sedative dose of xylazine followed by diazepam, ketamine was able to enhance the quality of anaesthesia for a short duration in donkeys (Abakar et al., 2014).

The combination of local with general anaesthesia is able to produces a cheapest, rapid, easily technique without side effects. The intratesticular and intrafunicular injection of local anesthetics attenuated the post castration pain by blocking of sensory nociceptors and reduction of nociception such as lidocaine and mepivacaine (Stucke et al., 2014) as it has a rapid onset, longer lasting activity, lower tissue irritation and relatively low toxicity (Lee et al., 2013).

Castration is a frequently performed operation in equine practice under effect of local or general anaesthesia. There are several studies that described the surgical techniques of equine castration such as (Mason et al., 2005, Sprayson and Thiemann, 2007) described the open castration with second intension healing, closed and half-closed castration with primary intention healing by scrotal or inguinal approach in standing or in dorsal or lateral recumbency. The inguinal approach castration with a primary intention healing in horses has a lower complication rate compared to the aforementioned techniques (Kummer et al., 2009). Therefore, this study also was performed to evaluate the cardiopulmonary and analgesic responses of xylazine, diazepam/ketamine and propofol infusion in donkeys that subjected to uncovered inguinal castration with a primary intension healing, and to evaluate the hypoalgesic effect scale of adding a local mepivacaine to the used anaesthetic protocol during castration by using of a visual analogue scale (VAS), Horse grimace pain scale (HGS) and composite pain scale (CPS).

2. MATERIALS AND METHODS

2.1. Donkeys

The study was performed on 10 male donkeys (Equus Asinus), age 10 ± 3 years old and weighed 145 ± 35 kg. Each donkey was placed in a warm, well ventilated and clean stable with free access to water and food. The study was approved by Animal Welfare and Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

2.2. Study design

A prospective, randomized and blinded study, all donkeys were allocated into two groups:

Xylazine, Ketamine, diazepam and propofol infusion (XDKP) group: Donkeys were castrated under TIVA.

Xylazine, Ketamine, diazepam, propofol and mepivacaine (XDKPM) group: Donkeys were castrated under TIVA in combined with mepivacaine (Mepecaine, Carpule Cartridges, Alexandria, 30 mg/ml). Two minutes before skin incision a 0.5 ml/cm of a local mepivacaine was infiltrated subcutaneously. The vaginal process was opened then the testes and spermatic cord were exposed. The mepivacaine 3% was infiltrated (2 ml/100kg) intratesticular and (1 ml/100kg) intrafunicular just distally to the supposed ligation location. Two minutes later, the spermatic cord was cut.

2.3. Experimental procedure

For all donkeys, the pre-anesthetic data were recorded including age, weight, body temperature, heart rate, respiratory rate, lung sounds, mucous membrane color, cecum sound and motility rate, skin hydration test and capillary refill time (CRT). Donkeys were fasting for 10 h before anaesthesia induction. One hour before anaesthesia induction, 10 mg/kg Gentamox (Amoxicillin 150 mg/ml and Gentamycin 40 mg/ml, Hipra, Spain) were administered by intramuscular (IM) injection and 1.1 mg/kg Flunixin (Flunixin meglumine, Norbrook, Ireland, 50 mg/ml) were intravenous (IV) injected. Donkeys were IV sedated with 1.1 mg/kg of xylazine (XylaJect; Adwia, Egypt; 20 mg/ml). Anaesthesia was inducted by IV injection of 2.2 mg/kg ketamine (Ketamine 5 mg/ml, Sigma-tec. Pharmaceutical Industries, SAE, Egypt) combined with 0.3 mg/kg diazepam (Neuril, Memphis, Egypt; 5 mg/ml). The score of anaesthesia induction was assessed as displayed in Table 1.

Once donkeys were in a lateral recumbency, they were lifted on a padded surgery table into a dorsal recumbency. Immediately, anaesthesia was maintained with an infusion of propofol at rate (0.1 mg/kg/min; Diprivan, 10 mg/ml, AstraZeneca, Egypt) to maintain anaesthesia by using an IV Infusion Set (100 ml, Burette set, Ultramed, Cairo, Egypt) connected with IV Flow Controller Extension Set (5 to 250 ml/hr, flow regulator, Ultramed, Cairo, Egypt). An eye ointment and a urinary catheter was placed before surgery in all donkeys. Invasive arterial blood pressures were measured every 5 min by placing a 22-gauge G catheter (Polypen, IV Catheter 22 G 25 mm, cannula without port, wings, indiamart, India) in the mandibular artery.

Inguinal castration was performed by one experienced surgeon according to (Sprayson and Thiemann, 2007). Briefly, the inguinal ring border was palpated and pushed the testes to over the external inguinal ring from scrotum through the inguinal canal. A 5-7 cm of the skin over the external inguinal ring was surgically incised and dissected of the underlying tissue layers. The

surgical opening was bluntly enlarged by the index of surgeon till exposed of tunica vaginialis. 5 cm of the partial layer of tunica vaginialis was incised and away from the external pudendal vein and Cremaster muscle. The two ends of incised tunica vaginalis were grasped to surgical opening side. A finger exploration of testes, epididymis tail, and proper ligament. Testes and spermatic cord were exteriorized to the surgical filed after cutting off the proper ligament. One transfixing ligation with one modified Miller's knot was applied at the closest point of the epididymis to the abdomen by using absorbable 2 USP suture material (EGYSORB, PGA Synthetic absorbable, Taisier-Med, Egypt). Avascular part of the spermatic cord was clamped, afterthought the spermatic cord was cut 1 cm distal to the ligation. After making ensure that the accurate ligation without hemorrhage, the clamp was removed and allowed to the cut end of the spermatic cord to pull into the abdomen. The surgical layers were sutured by using a simple continues suture pattern with 2/0 USP suture material then inguinal canal suture. Subcutaneous tissue layer was closed by using subcutaneous pattern 2/0 USP then the skin was stapled. Castration of the second testis was performed with the same procedures. The donkey's reflexes during the cutting of spermatic cord, cremaster muscle relaxation and over all the surgical conditions were recorded.

2.3. 1. Anaesthesia monitoring and assessment

Anaesthesia was monitored every 5 min by scoring qualitative parameters that indicative of anaesthesia depth: palpebral reflex, nystagmus and involuntary movements. Cardiovascular and respiratory variables were continuously monitored by a multi-parameter monitor (M69S user's manual, China) and recorded every 5 minutes: end-tidal expired carbon dioxide pressures (P_E'CO2), respiratory rate (RR, breaths per minute), heart rate (HR, beats per minute) and systolic arterial blood pressure (SAP), diastolyic arterial blood pressure (DAP) and mean arterial blood pressure (MAP) mmHg. The overall duration of anaesthesia was recorded (from initial ketamine administration to the end of protocol infusion).

2.3. 2. Recovery assessment

The recovery quality was scored according to Table 1. Donkeys were allowed to recover without assistance. During recovery, the time to sternal recumbences, the number of attempts to stand and the time taken to stand were recorded according to (Molinaro Coelho et al., 2014).

During recovery phase, cover of eyes should be done with complete quite surroundings till complete recovery occurred in all donkeys. Four hours postoperatively, the donkeys were IV administrated with phenylbutazone (PhenyloJect, 200 mg/ml, Adwia, Egypt) 4.4 mg/kg twice per day.

2.3. 3. Pain assessment

Scoring of pain was performed at the following time points: at baseline value T=0; (before the donkeys had received any drugs) and at T4, T8 and T24 h (post-surgical incision). Pain quantity and intensity were assessed by using two different pain scales. First one was the Horse grimace pain scale (HGS) according to (Dalla Costa et al., 2014) that has six items: stiffly backward ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin, strained nostrils and flattening of the profile. Each judgement point contains 3 grades from 0= no pain, 1= mild pain and 2= severe pain. The maximum total score is 12. Second one was the Composite Pain Assessment (CPS) according to (Bussieres et al., 2008). The scale has three divisions: a physiological division that contains 4 items (heart rate, respiratory rate, body temperature, digestive sounds), a behavioral division that contains 8 items of spontaneous behavior (posture, laying down, sweating, tail flicking, kicking of abdomen, pawing at floor, head movements, pain sounds) and an interactive division that contains 2 items (response to observer or reaction to palpation of painful area). Each item has four scores (0= no pain/normal behavior, 1= mild pain, 2= moderate pain, 3= severe pain). The maximum total score is 42.

2.4. Statistical Analysis

All statistical analyses were conducted using R studio (RStudio 0.99.903, © 2009-2016 RStudio, Inc.). Data were tested for normality using Kolmogorov-Smirnov test. Composite clinical scores that were shown to be normally distributed were analyzed by t-test and repeated measures outcomes were analyzed by repeated measures (ANOVA) for differences over time and between treatments.

Repeated measures general linear regression model was used to analyze composite pain score data with the different time points as the within-subjects factor and the treatment group as the between-subjects factor. Differences were considered significant when $P < 0.05$.

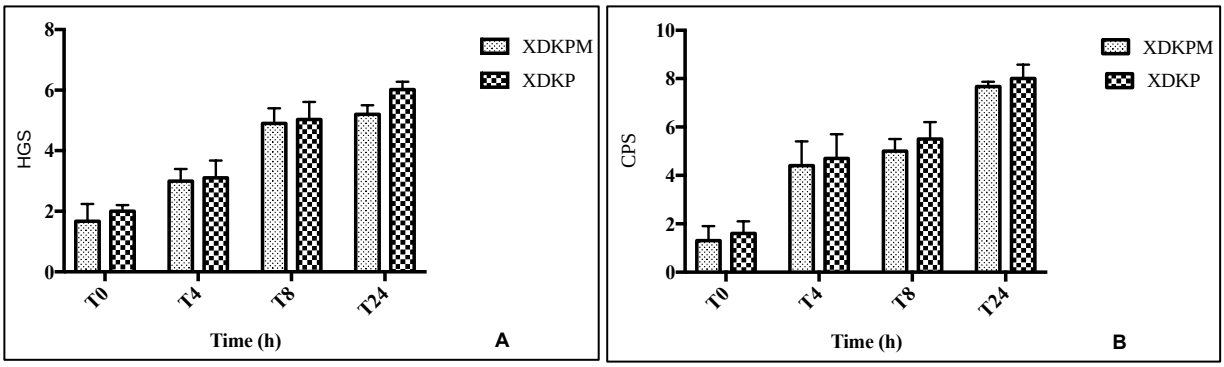

Figure 1: Mean and standard deviation of donkeys' pain scales (A: Horse Grimace Scale (HGS) and B: Composite Pain Assessment (CPS) at (before surgery) T0 and T4, T8, T24 (post-surgical incision) in XDKPM and XDKP groups.

Table 1: Induction and recovery scores

Criteria	Score
Induction	
Smooth induction without head or limb movement	Good=1
One or two steps before donkey falling to ground without paddling	Fair= 2
Ataxia and paddling with some danger to donkey	Poor= 3
Recovery score	
One to three attempts to stand without ataxia	Good=1
More than 3 attempts to stand with minimal short-term ataxia	Fair= 2
More than 3 attempts to stand with severe exciting or injuries	Poor=3

Table 2: Mean and standard deviation of donkeys' pain scales (Horse Grimace Scale (HGS) and Composite Pain Assessment (CPS) at (before surgery) T0 and T4, T8, T24 (post-surgical incision) in XDKPM and XDKP groups.

Time (h)	Groups	HGS	CPS
T0	XDKPM	1.7±0.5	1.3±0.6
	XDKP	2±0	2±0.5
T4	XDKPM	3±0.4[a]	4.4±1[a]
	XDKP	3.1±0.5[a]	4.7±1[a]
T8	XDKPM	4.9±0.5[a]	5 ±0.5[a]
	XDKP	5.03±0.6[a]	5.5±.7[a]
T24	XDKPM	5.2±0.3[a]	7.6±1.2[a]
	XDKP	6±1.2[a]	8 ±0.6[a]

[a] significant difference within the same group to (0) baseline $p<0.00$

3. RESULTS

3.1. Pre-anaesthesia evaluation:

There were no significant differences among the groups in age (XDKPM: 11.1±4.9; XDKP: 10.6±1.4) years, body weight (XDKPM: 126.3±3.2; XDKP: 138.7± 28.4) kg, body temperature (XDKPM: 37.6±0.1; XDKP: 37.5±0.4), HR (XDKPM: 36.6±8.1; XDKP: 36.6±6.9) beat/min, or RR (XDKPM: 18±2; XDKP: 17.3±1.2) breath/min, CRT <2 second and skin dehydration test <10 second of all donkeys before premedication of anaesthesia.

3.2.Anaesthesia induction

In both groups, anaesthesia induction was smooth. The induction doses of xylazine was sufficient for sedation of donkeys and no one of them needed more sedation dose. All donkeys were sternal recumbent 2.3 ± 1.2 min after injection of the sedative xylazine dose. The induction score was good=1 in all donkeys.

3.3.Anaesthesia monitoring parameters:

The mean range of the PE'CO2 was (XDKPM:42.1±0.2; XDKP:45.2±0.2) mmHg, SAP was (XDKPM:126.4±11.4; XDKP:122.6±18.8) mmHg, DAP was (XDKPM:61.2±3.8; XDKPM:68.9±8.9) mmHg, MAP was (XDKPM:83.5±5.9; XDKP:87.5±12) mmHg, RR

was (XDKPM:15.2±2.1; XDKP:18.6±1.3) bpm, and HR was (XDKPM:34.2±0.5; XDKP:36.2±0.4) bpm. These results showed no significant difference between the groups.

There was no statistically significant difference between the groups in the mean duration of the anaesthesia (XDKPM:60 ± 5; XDKP:62 ± 5.7) min. During a grasping of the spermatic cord and testes or spermatic cord ligation and cutting, all donkeys in XDKP group showed an increase in mean arterial blood pressure, while two of them showed limb movement reflexes.

3.4. Recovery assessment

The complete recovery occurred smoothly with mild ataxia. Recovery score ranged between good and fair (XDKPM:1±0; XDKP:1.3±0.6). The period of the recumbent position was (XDKPM:39±8.7; XDKP:36.3±2.3) min while, the period of the sternal position was (XDKPM:13.3±4.2; XDKP:12.3±2.5) min and the period of the standing position was (XDKPM:52.3±3.8; XDKP:48.6±1.6) min with no significant difference between groups.

3.5. Pain assessment scales

Preoperative facial expression of the donkeys in both groups was comma shape nostrils, disappeared sclerae and relaxed orbits, relaxed lips and non-prominent chins with relaxed chewing muscles, upward and forward directed ear position. No signs of restlessness or teeth grinding. Physiological and behavioural parameters were normal. No reaction was noted when testes were gently palpated.

Postoperative pain assessment: The donkeys in XDKP and XDKPM groups showed square-shape, widening of nostrils. Backward, downward and asymmetrical ear positions. Star shape eye and severe orbital tightening were further noted. Donkeys in both groups not revealed aggressive or kicking reaction when palpated in the surgical incision area. While, different degrees of tachycardia with tachypnea appeared. Pre-and post-operative pain behaviors in both groups such as sweating, tail flicking, rolling on the ground, abdominal kicking, the yawing behaviour or flehmen behaviour were not noted.

Both evaluated pain scales (HGS and EQUUS-COMPASS) were gradually increased and significantly differ ($p<0.05$) at T4, T8 and T24 h post-surgical incision within each group compared to baseline (T0) value. While there was no significant difference between both groups (Table 2; Figure 1).

4. DISCUSSION

This study designed to compare the effectiveness of two anaesthetic protocols DKXP alone or with the local mepivacaine DKXPM during castration in donkeys via inguinal approach. In addition to study the hypoalgesic effect of the multi-analgesic combination included xylazine as a preemptive analgesic and sedative agent then diazepam/ketamine during anaesthesia induction and propofol infusion during anaesthesia maintenance.

Assessing of pain in donkeys is not an easy task for many reasons: donkey is a stubborn and stoic animal; donkey does not respond to changes (pain or stress) like a horse; the observers should know that the casual or physical record is less than what is the donkey has (Matthews et al., 2002). Therefore, the current study designed to assess donkeys' pain depended on the most reliable and valid horse pain scale which was HGS and CPS.

The higher doses of drugs with short dosing intervals that used in the present study during treatment of donkeys attributed to the rapid metabolism and fast protein binding of these drugs in donkeys than horses also to avoid the depression of cardiopulmonary system following of inadvertent anaesthetic overdose. In addition, they obscured pain signs which make inadequate alleviation of pain (Matthews and van Loon, 2013).

The cardiopulmonary stability is a main goal during general anaesthesia with a sufficient analgesic that made a necessary to look for a regimen with minimal cardiovascular or respiratory depressions. The combination of ketamine with propofol is one of the perfect anaesthetic and analgesic regimen and suitable for induction and maintenance of anesthesia in donkeys and mules (Edner et al., 2002, Ohta et al., 2004, Molinaro Coelho et al., 2014).

Xylazine is an important sedative and licensed drug in the equine field in most countries. The known analgesic dose of xylazine in horses that produces excellent visceral analgesia is 1.1 mg/kg (Sanchez and Robertson, 2014) and in donkeys (Lizarraga and Beths, 2012).

Ketamine cannot be used as a sole anaesthetic agent during equine anesthesia because it has undesirable excitatory CNS effects (Bettschart-Wolfensberger et al., 1996) and faster metabolism in donkey which made the horse dose is insufficient to produce surgical plane. (Bettschart-Wolfensberger and Larenza, 2007) recorded an excellent induction score 1 when equine was inducted with ketamine and diazepam. For these reasons, it is necessary to add diazepam as well as to increase the induction doses of Ketamine/diazepam (2.2/0.3 mg/kg respectively) in donkeys, similar to (Matthews et al., 2005).

Propofol has a minimal cumulative effect and poor analgesic agent in donkeys and it is administrated

after premedication with xylazine is to produce a good quality of anesthesia (Matthews et al., 2002, Abd-Almaseeh, 2008, Naddaf et al., 2015). Using of propofol 1% as a sole anaesthetic agent was insufficient for induction, and it produced cardiopulmonary depression and a bad recovery in horses and also a large volume is required (Muir et al., 2009). Therefore, the use of propofol infusion is able to decrease its side effects (Matthews and van Loon, 2013, Molinaro Coelho et al., 2014).

Maintenance a balanced anaesthesia performed by combination between anesthetics with analgesics, (Bettschart-Wolfensberger and Larenza, 2007) used isoflurane and medetomidine while (Ringer et al., 2007) used isoflurane with lidocaine and in the previous studies in donkey evaluated the combination between alpha 2 agonists and propofol with or without ketamine (Molinaro Coelho et al., 2014, Naddaf et al., 2015).

Adding of Mepivacaine to the used combination is due to it has a less intrinsic vasodilator activity, faster onset of action, longer duration of action and possesses an intense motor nerve block ability (Lee et al., 2013). Additionally, (Stucke et al., 2014) reported that mepivacaine has a nociceptive effect during horse castration.

TIVA has a lower risk of hypotension and cardiopulmonary depression than inhalation anaesthesia in equine which explained a constant level of MAP between (73.5±4 and 87.5±1.1) mmHg in donkeys during castration (Naddaf et al., 2015).

The current results did not show an elevation of end tidal CO2 than 47 mmHg unlike (Naddaf et al., 2015) who showed a hypoxia. This may be due to injection of acepromazine which has a vasodilator and hypotensive properties. There was no respiratory depression or hypoxia occurred in this study, similar to (Naddaf et al., 2015, Umar et al., 2007). However, propofol is a hypotensive agent when bolus injected, and whereas not recorded during propofol infusion and it has a little effect on respiration (Edner et al., 2002, Ohta et al., 2004).

In the current study, HR showed non-significantly changes and the cardiovascular stability over anaesthesia period, dissimilar findings with (Naddaf et al., 2015) who permedicated with acepromazine and showed a significant increase of HR while (Abd-Almaseeh, 2008) who used propofol as a sole anaesthetic maintenance agent and showed a significant decrease of HR.

Because of the equine castration is painful visceral model (Sanchez and Robertson, 2014), HGS and CPS) significantly increased by time in both groups. On the other side, there was non-significant difference between DKXPM and DKXP groups in pain scales at different time points. This maybe because donkey is a unique different species than horses thus a specific grimace scale and other pain scale and it is able to hide pain sings (Ashley et al., 2005, Regan et al., 2016) or maybe the insufficient dose of mepivacaine.

5. CONCLUSION

On the basis of the results of this study, it could be concluded that XKDP is sufficient to produce a good quality of surgical anaesthetic plane and a degree of cardiopulmonary stability as well as profound the analgesic effect in donkeys. Adding of local mepivacaine to XKDP regimen did not attenuate increase of postoperative hypnogogic effect in donkey. HGS and CPS are potentially effective pain scales during castration in donkeys.

Further study should focus on designing the specific facial pain expression scales and the blood inflammatory mediators in donkeys at different multimodal analgesic protocols that may provide a base for the clinical use in various surgical interventions in donkeys.

7. REFERENCES

Abakar, J., Ghurashi, M., SERI, H. 2014. Evaluation of some anaesthetic protocols for induction of anaesthesia in donkeys (Equus asinus) in Sudan. J. Agric. Vet. Sci., 15: 1-15.

Abd-almaseeh, Z. 2008. Comparative anesthetic protocols: Propofol and thiopental in xylazine premedicated donkeys. J. Anim. Vet. Adv., 7: 1563-1567.

Ashley, F., Waterman-Pearson, A., Whay, H. 2005. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. Equine Vet. J. , 37: 565-575.

Bettschart-Wolfensberger, R., Larenza, M. P. 2007. Balanced anesthesia in the equine. Clin. Tech. Equine Pract., 6: 104-110.

Bettschart-Wolfensberger, R., Taylor, P., Sear, J., Bloomfield, M., Rentsch, K., Dawling, S. 1996. Physiologic effects of anesthesia induced and maintained by intravenous administration of a climazolam-ketamine combination in ponies premedicated with acepromazine and xylazine. Am. J. Vet. Res., 57: 1472-1477.

Bussieres, G., Jacques, C., Lainay, O., BeauchAMP, G., Leblond, A., Cadoré, J. L., Desmaizières, L-M., Cuvelliez, S. G., Troncy, E. 2008. Development of a composite orthopaedic pain scale in horses. Res. Vet. Sci., 85(2): 294-306.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M. C. 2014. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS. One., 9: 92281-92293.

Edner, A., Nyman, G., Essén,G., Avsson, B. 2002. The relationship of muscle perfusion and metabolism with cardiovascular variables before and after detomidine injection during propofol–ketamine anaesthesia in horses. Vet. Anaesth. Analg., 29: 182-199.

Friton, G. M., Philipp, H., Kleemann, R. 2006. Investigation of the clinical efficacy, safety and palatability of meloxicam (Metacam®) treatment in horses with musculosceletal disorders. Pferdeheilkunde., 22: 420-432.

Kummer, M., Gygax, D., Jackson, M., Bettschart-Wolfensberger, R., Fürst, A. 2009. Results and complications of a novel technique for primary castration with an inguinal approach in horses. Equine Vet. J., 41: 547-551.

Lee, h. M., Ok, S.-H., Sung, H.J., Eun, S. Y., Kim, H. J., Lee, S. H., Kang, S., Shin, I.-W., Lee, H. K., Chung, Y.K. 2013. Mepivacaine-induced contraction involves phosphorylation of extracellular signal-regulated kinase through activation of the lipoxygenase pathway in isolated rat aortic smooth muscle. Can. J. Physiol. Pharmacol., 91: 285-294.

Lizarraga, I., Beths, T. 2012. A comparative study of xylazine induced mechanical hypoalgesia in donkeys and horses. Vet. Anaesth. Analg. 39: 533-538.

Mason, B., Newton, J., Payne, R., Pilsworth, R. 2005. Costs and complications of equine castration: a UK practice based study comparing 'standing nonsutured'and 'recumbent sutured'techniques. Equine Vet. J., 37: 468-472.

Matthews, N., Taylor, T., Hartsfield, S. 2005. Anaesthesia of donkeys and mules. Equine Vet. Educ., 15: 102-107.

Matthews, N., Van Loon, J. 2013. Anaesthesia and analgesia of the donkey and the mule. Equine Vet. Educ., 25: 47-51.

Matthews, N.S., Taylor, T.S., Sullivan, J.A., 2002. A comparison of three combinations of injectable anesthetics in miniature donkeys. Vet. Anaesth. Analg., 29(1) 36-42.

Molinaro Coelho, C. M., Duque Moreno, J. C., Goulart, D. D. S., Caetano, L. B., Soares, L. K., Coutinho, G. H., Alves, G. E., Silva, L. A. F. 2014. Evaluation of cardiorespiratory and biochemical effects of ketamine propofol and guaifenesin ketamine xylazine anesthesia in donkeys (Equus asinus). Vet. Anaesth. Analg. 41: 602-612.

Muir, W., Lerche, P., Erichson, D. 2009. Anaesthetic and cardiorespiratory effects of propofol at 10% for induction and 1% for maintenance of anaesthesia in horses. Equine Vet. J. 41: 578-585.

Naddaf, H., Baniadam, A., Rasekh, A., Arasteh, A., Sabiza, S. 2015. Cardiopulmonary effects during anaesthesia induced and maintained with propofol in acepromazine pre-medicated donkeys. Vet. Anaesth. Analg. 42: 83-87.

Ohta, M., Oku, K., Mukai, K., Akiyama, K. M., Y 2004. Propofol-ketamine anesthesia for internal fixation of fractures in racehorses. J. Vet. Med. Sci. 66: 1433-1436.

Regan, F., Hockenhull, J., Pritchard, J., Waterman-Pearson, A., Whay, H. 2016. Identifying behavioural differences in working donkeys in response to analgesic administration. Equine Vet. J., 48: 33-38.

Ringer, S. K., KalchofneR, K., Boller, J., Fürst, A., Bettschart Wolfensberger, R. 2007. A clinical comparison of two anaesthetic protocols using lidocaine or medetomidine in horses. Vet. Anaesth. Analg., 34: 257-268.

Sanchez, L., Robertson, S. 2014. Pain control in horses: what do we really know? Equine Vet. J., 46: 517-523.

Sprayson, T., Thiemann, A. 2007. Clinical approach to castration in the donkey. In practice., 29: 526-531.

Stucke, D., Hall, S., Morrone, B., Gross Ruse, M., Lebelt, D. 2014. Different methods to identify pain after routine surgical castration of equine stallions: composite pain scale, facial expressions, faecal glucocorticoid metabolites and plasma cytokines. Equine Vet. J., 46: 2-5.

Umar, M. A., Yamashita, K., Kushiro, T., Muir W. W. 2007. Evaluation of cardiovascular effects of total intravenous anesthesia with propofol or a combination of ketamine-medetomidine-propofol in horses. Am. J. Vet. Res. 68: 121-127.