APPENDICES

APPENDIX A

ชื่อ.....ว/ด/ป.....

Quality	Grading	Score	Remarks
Sedation	**0 (ineffective sedation) = ไม่ซึม สนใจสิ่งแวดล้อมตลอดเวลา**	**นาทีที่ 1**	
	1 (mild sedation) = หัวตก สนใจสิ่งแวดล้อมเป็นระยะๆ ยืนมั่นคง วางน้ำหนักได้ทั้ง 4 ขา		
		2	
	2 (moderate sedation) = หัวตก ไม่สนใจสิ่งแวดล้อมหรือน้อยมาก เซแต่ยังพยุงตัวเองได้ด้วยขาทั้ง 4 ตลอดเวลา	**3**	
		4	
	3 (heavy sedation) = หัวตก ไม่สนใจต่อสิ่งแวดล้อมหรือน้อยมาก ไม่สามารถพยุงตัวด้วยขาทั้ง4/พิงซองบังคับ/พยายามล้มตัวลงนอน	**5**	
Ataxia	**0 (normal) = เดินตรงปกติ ขาทั้ง 4 รับน้ำหนักได้ดี ไม่เดินเซ**		
	1 (minimal ataxia) = เดินตรง>5 ก้าว แล้วเดินเซ ขาทั้ง 4 รับน้ำหนักได้ดี		
	2 (mild ataxia) = เดินตรง 1-2 ก้าว เดินเซชัดเจน แต่ยังรับน้ำหนักได้ทั้ง 4 ขา		
	3 (moderate ataxia) = เดินเซชัดเจน ตั้งแต่เริ่มเดิน แต่ไม่ล้ม		
	4 (very ataxia) = เดินเซมาก สามารถล้มเมื่อเดินเร็ว		
	5 (recumbent) = ล้มตัวลง ไม่สามารถรับน้ำหนักทั้ง 4 ขาได้เลย		
Induction	**0 (poor) = ตื่นเต้น เดินเซ พยายามลุกขึ้นและตะกุยขาอย่างรุนแรงจนอาจเป็นอันตรายต่อตัวล่อและผู้จับบังคับ**		

Quality	Grading	Score	Remarks
	1 (satisfactory) = เดินเซก่อนล้มลงนอน แสดงหรือไม่แสดงความพยายามที่จะลุกขึ้น มีตะกุยขาอยู่บ้างแต่ไม่รุนแรงมาก		
	2 (fair) = เดินเซก่อนล้มนอน ไม่พยายามลุก ตะกุยขาบ้างแต่น้อย		
	3 (good) = เดิน 1-2 ก้าวก่อนล้มตัวลงพื้น ไม่ตะกุยขา		
	4 (excellent) = ล้มตัวลงพื้นอย่างนุ่มนวลด้วยท่า sternal recumbency และค่อยๆ นอนท่า lateral recumbency ทางซ้าย		
Maintenance	**0** (poor) = เติมยาสลบ > 3 ครั้งหลังจากล้มลงนอนไป 20 นาที		
	1 (fair) =เติมยาสลบ 2-3 ครั้งหลังจากล้มลงนอนไป 20 นาที		
	2 (good) = เติมยาสลบ 1 ครั้งหลังจากล้มลงนอนไป 20 นาที		
	4 (excellent) = สลบอย่างราบรื่น ระดับ State 3 plane 1-2		
Recovery	**0** (Unable to stand) = มากกว่า 2 ชม.ไม่สามารถยืนได้ แต่พยายามหลายครั้ง ตื่นเต้น จนมีโอกาสเกิดบาดเจ็บ		
	1 (Poor) = พยายามยืนหลายครั้ง ตื่นเต้น มีโอกาสเกิดบาดเจ็บ		
	2 (Fair) = พยายามยืนมากกว่า 3 ครั้ง มีเดินเซมาก		
	3 (Satisfactory) = พยายามยืน 1-3 ครั้ง มีเดินเซ แต่ไม่ตื่นเต้น		
	4 (Good) = พยายามยืน 1 หรือ 2 ครั้ง เดินเซเล็กน้อย		
	5 (Excellent) = ยืนได้ตั้งแต่ครั้งแรก ไม่เดินเซ		

APPENDIX B

Anesthetic monitoring record

Date…………………………….

Signalment

Name DOB Age Breed
Sex Weight Body condition score

Physical examination (Before)

HR PR RR Temp.
CRT Mucous membrane Hydration status

Gut sound ABP Lung sound

เข้าซอง Stress/Nervous/Excited/Calm

Physical status I II III IV V Emergency
Catheter site **Duration of induction**
Procedure **Duration of surgery** **Duration of anesthesia**
Position
Fluid therapy Rate Volume
(Calculator………………….)
Distance from poll to floor Before After

PCV Hb TP
BUN AST

Time	Vital sign				Temp	MM	CRT	Reflex			Anesthetic agent/ Volume	Remark
	HR	PR	RR	MABP				Palpebral	Corneal	Nystagmus		

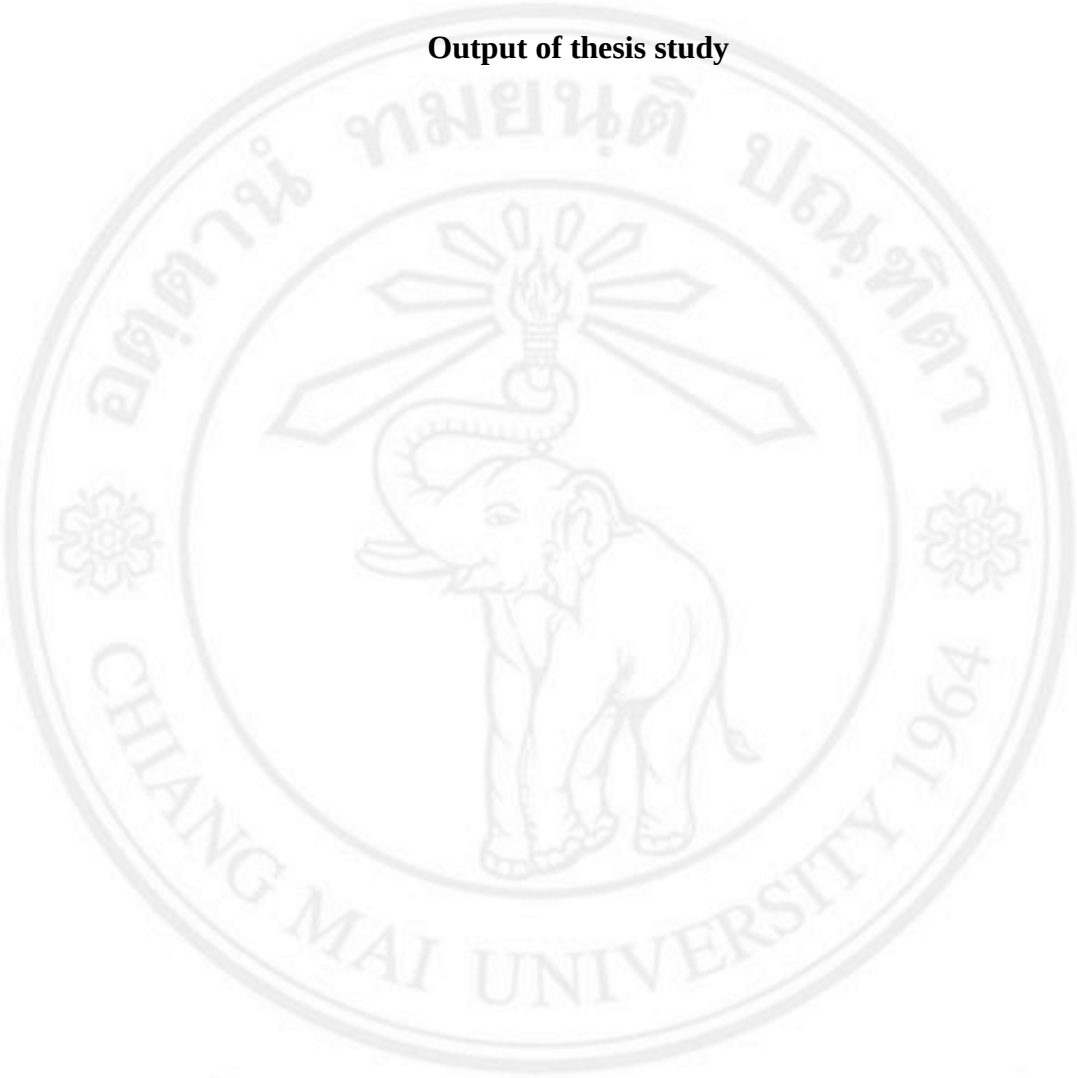
Recovery Time

1st movement of head/limb
Sternal recumbency without returning to lateral recumbency
Standing without returning to recumbency
No. attempting achievement of
-Sternal recumbency
-Standing
Stable/Normal gate/No ataxia
Urination

Remark

APPENDIX C

Output of thesis study



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Effects of Premedication with Xylazine and Detomidine on Quality of Thiopental Anesthesia for Castration in Mules

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Abstract

Clinical characteristics of premedication with xylazine and detomidine were compared in mules anesthetized for castration. Eighteen male mules were randomly premedicated with an intravenous injection (IV) of xylazine (1.6 mg/kg, n=9, group XY) or detomidine (0.03 mg/kg, n=9, group DET) before induction. Anesthesia was intravenously induced with thiopentone (6 mg/kg) and maintained its incremental IV dose (1.5-3.0 mg/kg) as needed. The qualities of sedation, anesthesia and recovery were graded and recorded. Heart, pulse, and respiratory rates and mean arterial blood pressure were recorded. All data was analyzed using Wilcoxon-ranksum test and Mann-Whitney *U* test. At 5 min after premedication, the mules showed moderate to heavy sedation and moderate ataxia in both groups. The induction was excellent and maintenance of anesthesia was fair. Following the induction, the mules of group XY collapsed faster than group DET (46.1±8.7 sec versus 76.3±18.4 sec, p=0.002). Recovery from anesthesia was smooth and uneventful in both groups. There was no significant difference in recovery score, duration of recovery, and number of attempts to sternal recumbency and standing between groups. There was no significant difference in heart, pulse, and respiratory rates and mean arterial blood pressure between groups although all vital signs in group DET were slightly higher than group XY. In conclusion, premedication using xylazine or detomidine for mules undergoing thiopental anesthesia were both satisfied. Both xylazine and detomidine are suitable for using premedicants before general anesthesia. Additional pain management which is as important for the proper sedation should be considered following 0.03mg/kg detomidine.

Keywords

Detomidine, Mule, Premedication, Thiopental, Xylazine

Introduction

The mule (*Equus mulus*) is a cross breeds between domestic donkey (*Equus asinus*) and domestic horse (*Equus ferus caballus*). Similar to horses, castration is performed under general anesthesia in mules because of safety for veterinarian and mules in addition to the animal welfare aspect[1-3].

Premedication using sedatives and/or analgesics has been known to improve the quality of general anesthesia and reduce the amount of anesthetic drugs required to produce adequate anesthesia[1, 4-10].

Alpha$_2$ adrenergic agonists (α_2 agonists) produce sedative, muscular relaxant and analgesic effects, and are commonly used in equine practice including xylazine and detomidine[6-14]. Detomidine produces more potent and longer sedation at lower doses than that of xylazine due to the difference of receptor binding and/or selectivity to the α_1 and α_2 adrenergic receptors[9]. Although there are a number of publications on α_2 agonists in horses, very few researches have been performed in mules. Differences in anatomy, behavior, physiology and also

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pharmacology amongst equine species are recognized. Thus, a protocol for horse anesthesia may not be entirely suitable for mules. It is also much more unpredictable with feral animals such as untrained-young mules. Variation of recommended dosage for α_2 agonists among equine species has been addressed[6, 11, 15-20]. Based on clinical practice, some practitioners indicated that approximately 1.5 times higher dosage of xylazine was required to produce adequate sedation in mules, compared to horses [15, 17-19]. Therefore, an intravenous dosage of 1.6 mg/kg xylazine or of 0.03 mg/kg detomidine has been recommended for premedication in mules[17]. Knowing the clinical efficacies of these drugs administered at the above dosages is crucial for a safe and effective premedication protocol. This study therefore aimed to compare the premedication effects of xylazine and detomidine in mules undergoing general anesthesia with thiopentone.

Methodology/Experimental design

Animals: Eighteen intact male mules, 1.5 to 3 years of age and weighing from 170 to 230 kg were castrated under general anesthesia. All mules were healthy and clinically free from cardiopulmonary diseases based on physical examinations. The mules were randomly divided into 2 groups that were premedicated with xylazine (group XY, n=9) or detomidine (group DET, n=9). This study was approved by The Ethic Committee for Laboratory Animal Usage, Faculty of Veterinary Medicine, Chiang Mai University.

Anesthetic protocols: The mules were premedicated with xylazine 1.6 mg/kg IV (Ilium xylazil-100®, Troy Laboratories Australia Pty Ltd, Sydney, Australia) or detomidine 0.03 mg/kg IV (Detomo vet injection®, Ceva Animal Health Pty Ltd, New South Wales, Australia). These dosages were employed based on previous reports [15, 17-19]. All of mules were anesthetized with thiopental sodium (Pentothal® 5% w/v, Jagsonpal Pharmaceuticals Ltd., Haryana, India) within 10 min after premedication when they were at least moderately sedated. Additional dosage of the two premedicants would be administered only if the mules were inadequately sedated. Surgical depth anesthesia required for castration was maintained with incremental IV dosages of thiopental. All mules were allowed to rest quietly after operation. The open technique of castration was applied to all mules in this study[3, 21].

Assessment of qualities of sedation and anesthesia: Scoring of the qualities of sedation, ataxia and anesthesia was summarized in Table 1. The quality of sedation following the premedication was evaluated using sedation and ataxia scores (Table 1) and head lowering ratios during the first 5 min after premedicated with xylazine or detomidine. The sedation and ataxia scores used in this study were modified from the previous reports in horses[22, 23, 24]. The head lowering ratios were retrieved from video recorded images and analyzed using Tracker ver.2.73 software (written by Douglas Brown, available: http://www.cabrillo.edu/~dbrown/tracker/). Lateral canthus of the left eye was used as a landmark for determining and head height was measured a distance from the lateral canthus to the floor. The head lowering ratios were an alteration in the level of the head carriage from the ground, which can be calculated from the height of lateral canthus after premedication divided by the height before premedication.

The qualities of anesthesia were evaluated using induction, maintenance, and recovery scores (Table1). These scores were modified from the previous reports conducted with horses[25-26]. In additional, total IV amount of thiopentone for induction and maintenance of anesthesia, duration of induction, maintenance period, time to the first movement, duration of recovery, and number of attempts to sternal recumbency and to standing were recorded. The duration of induction was defined as duration from the end of the first IV administration of thiopentone to achieving lateral recumbency. The maintenance period was defined as duration from the achieving lateral recumbency to finishing operation. The durations of recovery were divided into 3

steps, first; started from the last IV administration of thiopentone and ended when the mule showed the first gross movement, second; time of first movement to achieving sternal recumbency without returning to lateral recumbency, and finally; time of sternal recumbency to standing. The number of attempts to accomplish each task was also recorded.

Table 1 Criteria for scoring the qualities of sedation, ataxia, anesthetic induction, maintenance of anesthesia and recovery from anesthesia in mules.

Score	Criteria
Sedation	
0 (Poor)	Fully respond to surrounding stimulants.
1 (Mild)	Slightly lowering the head but still respond to the surrounding stimulants.
2 (Moderate)	Lowering the head. The muzzle is below carpus. No response to environment. All limbs are still bearing weight.
3 (Heavy)	Lowering the head. No response to environment. Lean to the restrain box or fall down.
Ataxia	
0 (Non ataxic)	Normal walking gait. All limbs can bear weight.
1 (Minimal)	Normal walking gait for at least 5 steps but ataxia later. All limbs can bear weight.
2 (Mild)	Only 1-2 normal walking gait and then ataxia.
3 (Moderate)	Ataxia since the first step but no sign of falling down.
4 (Very ataxic)	Ataxia since the first step. Likely to fall down when walk faster.
5 (Recumbent)	Fall down. Cannot bear weight.
Induction	
0 (Poor)	Ataxia, excited and pawing. Danger to the mules and handlers.
1 (Fair)	Ataxia and paddling with/without trying to stand up.
2 (Satisfactory)	Ataxia with/without paddling after falling down.
3 (Good)	Moved 1 or 2 steps with no paddling after falling down.
4 (Excellent)	Smoothly fall down to the ground.
Maintenance	
0 (Poor)	Multiple incremental bolus IV doses of thiopentone required during the first 20 min.
1 (Fair)	Top up 2-3times of 1.5-3 mg/kg thiopentone required after first 20 min.
2 (Good)	Top up 1times of 1.5-3 mg/kg thiopenonel required after first 20 min.
3 (Excellent)	Smoothly maintenance without thiopentone increments.
Recovery	
0 (Unable to stand)	Mule cannot stand for >2 hours after multiple attempts to stand; excitement; injury or high risk of injury
1 (Poor)	Multiple attempts to stand; excitement; high risk of injury
2 (Fair)	Stands > 3 attempts; substantial ataxia
3 (Satisfactory)	Stands after 1 to 3 attempts; prolonged ataxia but no excitement
4 (Good)	Stands after 1 or 2 attempts; mild ataxia
5 (Excellent)	Stands after first attempt; no ataxia

Cardiopulmonary monitoring: Heart rate (HR), pulse rate (PR), respiratory rate (RR) were recorded a day before operation as baselines. On the experiment day, these parameters were recorded within 10 min after induction onward[27]. Indirect mean arterial blood pressure (MABP) was measured by using the cuff at undertail-covert. The data of HR, PR and RR were presented every 5 min in percentage to the baselines of each mule. The data of MABP was shown every 5 min in mmHg unit as there was no baseline available.

Statistical Analysis: Data are shown in mean ± standard deviation (S.D.) or mode and range. Comparisons of parameters between group XY and DET were tested by Wilcoxon-ranksum test for the continuous data and Mann-Whitney *U* test for ordinal data using the STATA 9.2 software. $P<0.05$ was considered statistical significance.

Results and Discussion

The study was conducted with mule stallions undergoing castration as it was a repeated, standardized procedure which allowed comparison of the two drugs. The age and body weight between groups were not differences (Table 3).

Quality of sedation following premedication: Central α_2 adrenoceptors, once activated by their selective agonists, decrease norepinephrine release leading to CNS depression[5]. Sedative effect of these drugs benefits in reduction of anesthetic consuming and side effects in veterinary practice. Detomidine is much more selective to α_2 adrenergic receptors than xylazine[28], hence; it has more potent in sedation and analgesia[29]. At equivalent sedative dose to xylazine, detomidine is known for its longer lasting effect[11]. This is true when used in horses. In this study, dosages of both α_2 agonists for mules were assigned based on 1.5 times of those recommended with equality of their sedative action in horses[30]. Following the premedication, the mules showed signs of

sedation, including lowering their head and ataxia. Our results revealed non-significant difference in sedation and ataxia scores between the two drugs (Table 2). The head lowering ratios result in this study was in agreement with a previous report[31] that group XY dropped the head lower than group DET especially at two min after administration (p=0.03) (Table 2).

Table 2 Qualities of sedation following the intravenous administration of xylazine (1.6mg/kg; n=9) or detomidine (0.03mg/kg; n=9) as a premedication in mules.

	Minutes after the premedication				
	1	2	3	4	5
Sedation score (0-3)					
Xylazine	1 (0-3)	2 (1-3)	3 (2-3)	3 (2-3)	3 (2-3)
Detomidine	1 (0-2)	2 (1-3)	2 (2-3)	2 (2-3)	2 (2-3)
Ataxia score (0-5)					
Xylazine	NA	NA	NA	NA	3 (1-3)
Detomidine	NA	NA	NA	NA	3 (0-3)
Head lowering ratio					
Xylazine	0.916	0.619*	0.519	0.512	0.499
Detomidine	0.906	0.739*	0.628	0.600	0.574

* Significant difference (P<0.05) between groups premedicated with xylazine and detomidine. Sedation and ataxia score were shown in mode (range). Head lowering ratio was shown in ratios of lateral canthus height after premedication (A) divided by the height before premedication (B) (A/B).

Qualities of anesthesia and recovery: All mules showed sufficient moderate sedation, therefore, no mules received additional dose of xylazine or detomidine before the IV administration of thiopentone. Although 2 mules of group XY and a mule of group DET showed poor induction, the rest were smoothly anesthetized with the IV administration of thiopentone. There was no significant difference in the induction score (Table 3). The induction time of group XY was half a minute shorter than that of group DET (p=0.002) (Table 3) but this was not clinically significant to the author's point of view. Maintenance score and the total amount of thiopentone administered to mules were not significantly different between groups (Table 3). However, 5 mules of group XY and 2 mules of group DET received total amount of thiopentone exceeding the dose limit for horses (11 mg/kg[10]) to maintain at the stage III plane 2 of general anesthesia[9] but this did not consequently affect to the recovery quality.

Recovery from anesthesia was uneventful and clinically acceptable in all mules. There was no significant difference in recovery score, duration of recovery, and numbers of attempts to sternal recumbency and standing between groups (Table 3).

Table 3 Qualities of thiopental anesthesia following the premedication with an intravenous xylazine (1.6mg/kg; n=9) or detomidine (0.03mg/kg; n=9) in mules.

	Premedicants		p value
	Xylazine	Detomidine	
Age (months old)	24.2±4.5	27.1±4.2	0.143
Body weight (kg)	190.6±24.2	210.9±24.3	0.156
Total amount of thiopentone (mg/kg)	2,384.8±597.6	2,392.5±903.6	0.627
Induction score	4 (0-4)	4 (0-4)	0.575
Maintenance score	1 (1-2)	1 (1-2)	0.125
Recovery score	4 (2-4)	4 (2-4)	1.000
Induction time (sec)	46.1±8.7	76.3±18.4	0.002
Maintenance period (min)	33.7±10.4	30.9±11.1	0.402
Recovery times			
First movement (min)	24.9±9.9	30.3±6.9	0.171
Sternal recumbency (min)	39.8±10.4	46.8±10.3	0.135
Standing (min)	50.7±14.2	59.8±17.5	0.269
Number of attempts			
to sternal recumbency (times)	1 (1-3)	1 (1-8)	0.886
to standing (times)	1 (1-10)	2 (1-4)	0.612

All data were shown in mean ± S.D. or mode (range).

Changes in cardiopulmonary parameters during general anesthesia: Changes in cardiopulmonary system are one of the major adverse effects of α_2 agonists reported in mammals. Activating α_2 adrenergic receptors centrally and presynaptically reduces sympathetic tone and subsequently causes bradycardia and hypotension[32-34]. CNS depression after administration of α_2 agonists causes bradypnea[32-34]. In our study, the baseline values of HR, PR and RR were measured while the mules were in the resting stage a day before the operation. It was very difficult to measure indirect MABP because of the mule's excitement with the procedure at their rear and it was impossible to measure direct MABP during their consciousness. Thus there was no MABP baseline data in our study. All vital signs were also unable to perform

during sedation stage as the procedures would interfere the sedation quality and the consequent anesthetic induction. The cardiovascular results were, therefore; obtained only under unconscious condition. Changes in HR, PR, RR (percentage to their own baselines) and MABP during general anesthesia for castration were shown in Fig 1. The time point that a mule fell down after thiopental induction was assigned to Minute 0.

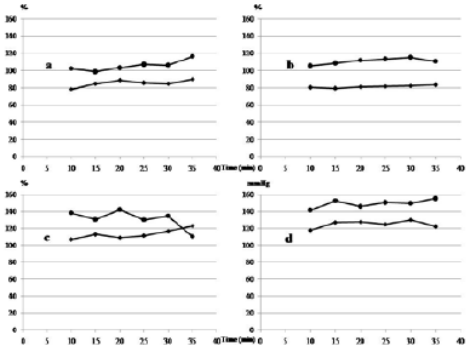

Fig.1 Percentage changes to the baselines of heart rate (a), pulse rate (b), respiratory rate (c), and changes of mean arterial blood pressure (d) under general anesthesia between mules premedicated with xylazine (♦) and detomidine (•). Minute 0 is the time once mules fall down after induction.

Whilst xylazine induced more profound bradycardia, group DET had no such an effect but, instead, slightly tachycardiac and tachypneic. Because of unavailable baseline MABP data, it is impossible to define whether the mules were in hypo- or hypertension. However, when compared to the awaken horse MABP[35], our anesthetized mules were seemed to be hypertension, especially in group DET that had higher MABP than group XY at every time point. These are not all in agreement to the previous reports in horses that both xylazine and detomidine suppress cardiopulmonary function[22, 36]. However, all vital signs data of group DET were slightly higher than group XY during the operation but there was no significant difference between groups at any time points. The elevation of these parameters in the group DET could result from, firstly, stimulation of α_1 receptors in case of excessive doses of the α_2 agonists and secondly, visceral pain induction during the operation. To the authors' view, the dosage of detomidine (0.03 mg/kg) in this study was not too excessive as it is still in the range that was acceptable to use in horses while xylazine dosage was far more than the recommendation for horses. Thus the activation of α_1 adrenergic receptors in this case is not yet reasonable. The author would rather go for the latter explanation. It was possible that castration procedure without local or regional anesthesia caused visceral pain that overcame analgesic effect of detomidine at this dosage.

Conclusion

Xylazine at 1.6 mg/kg and detomidine at 0.03 mg/kg delivered satisfied and comparable premedication effects for thiopental anesthesia in mules. Detomidine at this dosage apparently had no cardiopulmonary suppress but perhaps not as good in analgesia as xylazine based on the increasing of cardiopulmonary parameters during the operation. Higher premedicative dosage of detomidine should be further investigated to find an optimum point for a better analgesia with minimal cardiopulmonary effect. Another option is combination of other protocols for pain management concomitant to α_2 agonists premedication.

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