CHAPTER III

MATERIALS AND METHODS

1. Study subjects

Eighteen healthy intact male mules, the cross breds between Thoroughbreds or Thoroughbred/Quarter horse cross-breds and Australian Mammoth or Teng-zhou donkeys, aged between 1.5 to 3 years old and weighed from 170-230 kg were recruited from a mule stud farm in Chiang Mai province for this study. All of the mules were raised in similar management and were to be castrated as a routine for military purposes. All of them were healthy and clinically free from cardiopulmonary, liver, and renal diseases based on the results of a physical examination and the blood test prior to the experiment. Hematology and serum chemistries including complete blood count (CBC), blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), and total plasma protein (TP) were performed at the Veterinary Diagnostic Laboratory, Chiang Mai University. The surgical area is well-ventilated indoor with straw bedding near the mule's box. The experiment was performed in June until October 2011 which is rainy season and all mules were operated only in the morning.

The experiment was designed as clinical trial. The mule subjects were randomized into two groups receiving single dose of either xylazine or detomidine using double-blind technic. The numbers of subject were calculated by Winepiscope with 95% level of confidence, 80% power of test, 100 proportions in population 1 and 60 proportions in population 2. The mules were randomly divided into 2 groups,

group one was assigned for xylazine premedication (group XY, n=9) and group two was assigned for detomidine premedication (group DET, n=9). The experiment was conducted in a field practice basis. The research has been approved by The Ethic Committee for Laboratory Animal Usage, Faculty of Veterinary Medicine, Chiang Mai University.

Inclusion criteria of subjects in the study

1. Both testis of the mules must be completely descended into the scrotum and located in vertical midline (2).

2. Physical status for anesthetic risk is in class 1 (minimal risk) for anesthesia (5).

3. Hematology must be in the normal referral range; see below: (59)

3.1 Pack cell volume (PCV) = 30.2 – 34.3%

3.2 Hemoglobin (Hb) = 10.4 – 11.9 g/dL

3.3 Red blood cell count (RBC count) = 5.8 – 6.5 x 10^6 cells/µL

3.4 White blood cell count (WBC count) = 10,600 – 13,100 cells/µL

3.5 Platelet cell count: 197,000-287,000 cells/µL

4. Blood chemistry must be in the referral range; see below: (59)

4.1 Total plasma protein = 6.0 – 6.7 g/dL

4.2 Aspartate aminotransferase (AST) = 231 – 303 unit/L

4.3 Blood Urea Nitrogen (BUN) = 34 – 43 mg/dL

4.4 Creatinine (Cr) = 1.03 – 1.2 mg/dL

5. Negative to Equine Infectious Anemia Virus (EIAV) confirmed by Coggins' test.

6. Negative to blood parasite confirmed by thin blood smear.

7. Vital signs were within normal range; see below: (22).

7.1 Heart rates (HR) = 35 – 60 beats/minute

7.2 Pulse rates (PR) = 30 – 45 beats/minute

7.3 Respiratory rates (RR) = 8 – 20 breaths/minute

7.4 Temperature = 99.5 – 101.0 °F

7.5 No sign of cardiac murmur

7.6 Clear lung auscultation

7.7 Pink mucous membrane

7.8 Capillary Refill Times (CRT) must be less than 2 seconds.

8. No enlarged submandibular lymph nodes

9. No evidences of abnormality such as inappetite, nasal discharge, coughing, respiratory noise, polydipsia, and polyuria.

Exclusion criteria of subjects in the study

1. Receiving of CNS depressing drugs other than thiopental sodium during the maintenance period of anesthesia.

2. Receiving of additional dosage of the premedicants

3. The operation time longer than 1 hr.

4. Having live threatening signs during anesthesia; see below:

4.1 Blood pressure < 60 mmHg (5, 60)

4.2 Cardiac murmur

4.3 Excessive bleeding

4.4 Hypoxemia

2. Experiment:

Pre-experiment records and evaluations

Signalments, including name, age, breed, body weight, and body condition score of all mules were recorded. Every mule was acclimatized to the physical restrain procedures and place being used in the study for 7 days prior to the initiation of the experiment. The acclimatization procedures include haltering, hand walking, entering a restrain box, and standing in a restraining box. The restraining box was made from iron pipe. An external diameter was 2.5 inches. The restrain box dimension was 100 cm in width, 168 cm in length, and 139 cm in height. The mules were trained to stay inside the restraining box for 5 min daily for one week. Blood collection for hematology study was performed 7 days before the experiment, including serum chemistry, EIAV, and blood parasite checks.

One day before the experiment, each mule was randomly assigned into two groups, xylazine or detomidine. The physical examinations were performed to evaluate physical status and anesthetic risk and record the baseline values for cardiopulmonary monitoring. Heart rate (HR), pulse rate (PR) and respiratory rate (RR) were obtained by auscultation and palpation. Rectal temperature was measured using digital thermometer. Capillary refill time (CRT) and mucous membrane color were evaluated at the gum above one of the corner incisors. Hydration status was evaluated by skin pinch test over the shoulder. At this day, the mule's head height at rest was also recorded using a digital VDO camera. All mules were withheld from food and water for 12 hr before anesthesia.

Preparation of medical administration site

On the day of the experiment, right external jugular vein was prepared by clipping the hair and scrubbing around that area with aseptic technic. A 16-gauge, 2-inch long intravenous catheter (NIPRO®, Nipro Medical Corp., Miami, U.S.A.) was inserted into the right external jugular vein. The catheter was fixed to the skin by using epoxy glue and adhesive tape.

Anesthetic process and monitoring

- **Premedication**

Mule's behavior prior to premedication was recorded as excited, frightened, stress or calm. The mules were administered intravenously with either 1.6 mg/kg BW xylazine (Ilium xylazil-100®, Troy Laboratories Australia Pty Ltd, Sydney, Australia 100mg/ml) or 0.03 mg/kg BW detomidine (Detomo vet injection®, Ceva Animal Health Pty Ltd, New South Wales, Australia 10mg/ml). These dosages were employed based on previous reports (18-21). All research staff was blinded to the type of premedicants administered to each mule.

Sedation parameters were evaluated and recorded including sedation score, ataxia score, and head lowering ratios at min 1 to 5. The technic of head lowering measurement is explained in figure 7 using digital VDO camera and analyzed by Tracker ver.2.73 software (written by Douglas Brown, available: http://www.cabrillo. Edu/~dbrown/tracker/). Sedation and ataxia score were evaluated by at least 3 experienced veterinarians according to criteria explained in table 1 (61, adapted from62, adapted from63). Before the experiment, the experienced veterinarians were standardized for scoring the qualities of sedation, ataxia, induction, maintenance and recovery.

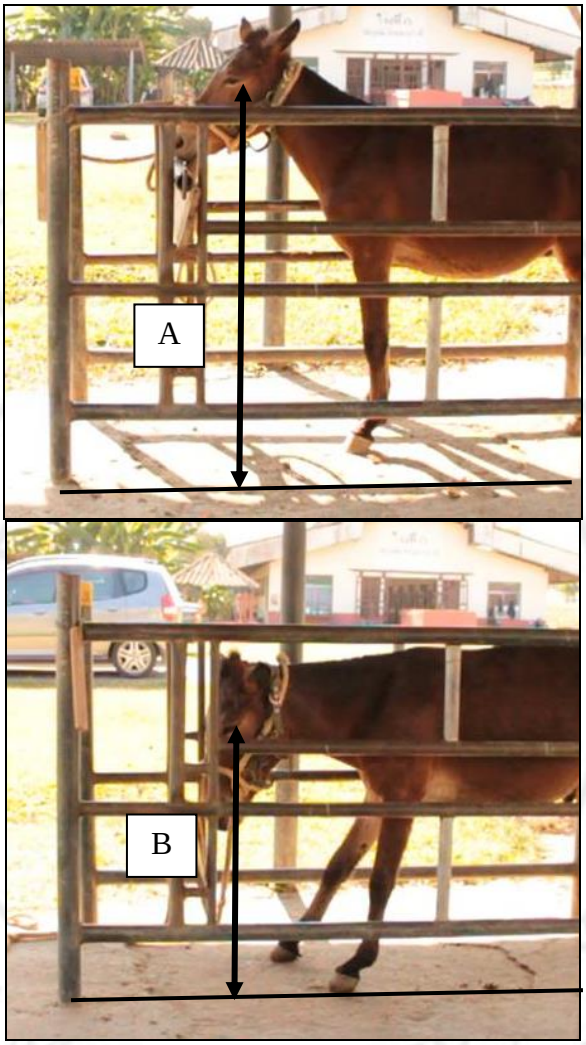

Figure 7. The technic of head lowering ratio measurement. The head height at rest before premedication was demonstrated at the left and after premedication at the right. Lateral canthus of the left eye was used as a landmark for determining an alteration in the distances of the head carriage from the ground before (A) and after (B) premedication. The head lowering ratio can be calculated from the height of lateral canthus after premedication (B) divided by the height before premedication (A) (head lowering ratio = B/A). The measurement and calculation were repeated three times and the data was reported an average value.

Table 1. Criteria for scoring the qualities of sedation, ataxia, anesthetic induction, maintenance of anesthesia, and recovery from anesthesia in mules (61, adapted from62, adapted from63)

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.

Table 1. (continued) Criteria for scoring the qualities of sedation, ataxia, anesthetic induction, maintenance of anesthesia, and recovery from anesthesia in mules (61, adapted from62, adapted from 63)

Score	Criteria
Anesthetic 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 of anesthesia	
0 (Poor)	Multiple incremental bolus IV doses of thiopentone required during the first 20 min.
1 (Fair)	Top up 2-3 times of 1.5-3 mg/kg thiopentone required after first 20 min.
2 (Good)	Top up 1 times of 1.5-3 mg/kg thiopentone required after first 20 min.
3 (Excellent)	Smoothly maintenance without thiopental increments.

Table 1. (continued) Criteria for scoring the qualities of sedation, ataxia, anesthetic induction, maintenance of anesthesia, and recovery from anesthesia in mules (61, adapted from62, adapted from 63)

Score	Criteria
Recovery from anesthesia	
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

- **Induction**

The mules were leaded to the operation area and thiopental induction was then performed only when mules were moderately sedated (at least sedation score 2) within 10 min after premedication by the same anesthetist throughout the experiment. Thiopental sodium (Pentothal®, Jagsonpal Pharmaceuticals Ltd., Haryana, India sterile powder 1 g; solution 5% w/v) 6 mg/kg BW was infused through the catheter. The first half of the drug was infused rapidly following by slow infusion of the second half. And the mules were physically restrained to left lateral recumbency. The quality of induction were scored (Table 1) by three experienced veterinarians. The duration of induction was defined as duration from the end of the first IV thiopental administration to achieving lateral recumbency.

- **Maintenance**

Once the mules were in the lateral recumbency, normal saline solution was administered intravenously though the catheter at a rate of 10 ml/kgBW/hr during anesthesia. Anesthesia was maintained at stage 3 plane 2 with incremental IV dosages (2-3 mg/kg BW) of thiopentone. The castration was operated within 1 hr. There are 2 surgeons in this experiment whose experience more than 20 years. The maintenance period was defined as duration from the achieving lateral recumbency to the completion of surgical procedure. Vital signs were monitored throughout the operation. The quality of maintenance was scored (Table 1) by an anesthetist during the first 20 min after lateral recumbence. Cardiopulmonary parameters and body temperature were recorded every 5 min after lateral recumbency using the following equipment:

1. Heart rate: stethoscope (3M™ Littmann® Classic II S.E.)
2. Pulse rate and oxygen flow rate: pulse oximeter (Biosys®)
3. Body temperature: temperature probe (Biosys®)
4. Indirect mean arterial blood pressure (Indirect MABP): the 210x73 mm cuff (Biosys®) was stripped at undertail-covert
5. Capillary refill time and mucous membrane color: pressing on the mucous membranes at the corner incisor

The data of HR, PR and RR were presented as percentage to the baselines of each mule. The data of indirect MABP was shown every 5 min in mmHg unit as there was no baseline available.

The optimum depth of anesthesia (stage 3 plane 2) was evaluated subjectively by an anesthetist according to

1. The responses of HR, PR and RR: reduce the rates but still regular
2. Palpebral reflex: disappear of the mule blink when touch the corner of the eye
3. Corneal reflex: depression of the mule blink when touch the cornea of the eye
4. Nystagmus reflex: disappear of nystagmus sign (involuntary of eye movement)
5. Eye position: the eyeball become the central
6. Jaw tone : no muscle tone that can open the mouth without resistance

Dobutamine hydrochloride (Dobutamine injection® concentration 250 mg/20 ml, U SQUARE LIFESCIENCE PVT.LTD, Gujarat, India) was prepared

just in case of hypotensive mule (MABP < 60 mmHg) by slow IV infusion 0.5-1.0 µg/kg/min until MABP was greater than 75 mmHg (5, 60).

Surgical procedure of castration

Method of operation in this experiment was opened-castration on healthy intact male mules (2, 64). The mules were positioned in left lateral recumbency. Their upper hindlimb were pulled forward and tied around their neck by a nonslip loop. Clipping and cleaning the surgical area by aseptic technique. A mule was operated by the experienced surgeons. Each testis was squeezed ventrally in the scrotum. The scrotum was incised pole-to-pole parallel though the skin and dartos parallel to median raphe with a scalpel. The incisor was 1 cm from the median raphe. The scrotal fascia was removed around the vaginal tunic. The parietal tunic of the testis was incised and the testis was exposed. The ligament of the tail of the epididymis was blunted. The mesorchium was transected so the testis, epididymis and distal part of spermatic cord were completely separated from the parietal tunic that removed by using emasculators. Duration of surgery (considering from induction to finishing operation) must be less than an hour. After operation, the mule was injected intramuscularly with tetanus antitoxin (Tetanus Antitoxin B.P. ® 1,500 units per bottle concentration, Serum Institute of India, Ltd., India) 3,000 units, penicillin G sodium (Penicillin G® 5,000,000 units per Vial, General Drugs House Co., Ltd., Bangkok, Thailand) 25,000 units/kgBW IV, gentamicin sulphate (Gentafar 100® 100 mg/ml concentration, Eurovet Animal Health B.V., The Netherlands) 4.4 mg/kgBW and phenylbutazone (Butasyl® 186.1 mg/ml concentration, Fort Dodge Veterinaria , S.A., Spain) 4.4 mg/kgBW IV.

- **Recovery**

Recovery began when thiopental administration ceased. All mules were allowed to rest quietly after the completion of surgical procedures. The monitoring machine and fluid set were removed when the first movement was observed. The durations to recovery were divided into 3 periods, first; started from the last IV administration of thiopentone to the first gross movement of the mules, second; time of first movement to achieving sternal recumbency without returning to lateral recumbency, and finally; time of sternal recumbency to standing. The durations of each period were recorded and the number of attempts to accomplish each task was also recorded. The number of handlers who supported a mule during recovery was limited to 3 persons to avoid unnecessarily disturbance to the mule.

Post operative care

Post-operative care included infection prophylaxis and inflammation control. Mules were intravenously administered with 25,000 units/kgBW penicillin G sodium once a day for 7 days and 4.4 mg/kgBW gentamicin once a day for 7 days. Mules were also administered with 2.2 mg/kgBW IV doses of phenylbutazone once a day for 2-3 days subjected to the condition of the surgical wounds.

3. Statistical Analysis

The data were 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, Mann-Whitney *U* test for ordinal data using the STATA 9.2 software. $P < 0.05$ was considered statistical significance.