A COMPARISON OF KETAMINE COMBINED WITH DEXMEDETOMIDINE AND KETAMINE COMBINED WITH XYLAZINE ON ANALGESIA IN RABBITS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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การเปรียบเทียบผลการใช้เคตามีนร่วมกับเด็กซ์เมทดีโตมิดีนและเคตามีนร่วมกับไซลาซีนในการระงับความปวดในกระต่าย

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Thesis Title	A COMPARISON OF KETAMINE COMBINED WITH DEXMEDETOMIDINE AND KETAMINE COMBINED WITH XYLAZINE ON ANALGESIA IN RABBITS
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ออมอุสาห์ กัวหา : การเปรียบเทียบผลการใช้เคตามีนร่วมกับเด็กซ์เมทดีโตมิดีนและเคตามีนร่วมกับไซลาซีนในการระงับความปวดในกระต่าย. (A COMPARISON OF KETAMINE COMBINED WITH DEXMEDETOMIDINE AND KETAMINE COMBINED WITH XYLAZINE ON ANALGESIA IN RABBITS) อ.ที่ปรึกษาหลัก : ผศ.น.สพ. ดร.สุมิตร ดุรงค์พงษ์ธร, อ.ที่ปรึกษาร่วม : ผศ.น.สพ. ดร.ธวัช เล็กดำรงศักดิ์

การศึกษานี้เปรียบเทียบผลการใช้เคตามีนร่วมกับเด็กซ์เมทดีโตมิดีนและเคตามีนร่วมกับไซลาซีนในการระงับความปวดต่อระบบหมุนเวียนเลือดและการหายใจในกระต่ายพันธุ์นิวซีแลนด์ไวท์ที่มีสุขภาพดี การทดลองใช้วิธีการสุ่มเลือกแบบดับเบิ้ลครอสโอเวอร์ กระต่ายถูกแบ่งแบบสุ่มออกเป็น 2 กลุ่ม กลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน (จำนวน 4 ตัว ตัวผู้ 2 ตัว ตัวเมีย 2 ตัว) ได้รับยาเคตามีน 35 มิลลิกรัมต่อกิโลกรัมและยาเด็กซ์เมทดีโตมิดีน 0.5 มิลลิกรัมต่อกิโลกรัม ใต้ชั้นผิวหนัง และกลุ่มยาเคตามีนร่วมกับยาไซลาซีน (จำนวน 4 ตัว ตัวผู้ 2 ตัว ตัวเมีย 2 ตัว) ได้รับยาเคตามีน 35 มิลลิกรัมต่อกิโลกรัมและยาไซลาซีน 5 มิลลิกรัมต่อกิโลกรัม ใต้ชั้นผิวหนัง ค่าพารามิเตอร์ทางสรีรวิทยา ประกอบไปด้วย อัตราการเต้นของหัวใจ อัตราการหายใจ อัตราชีพจร เปอร์เซ็นต์ออกซิเจนอิ่มตัวในเม็ดเลือดแดง ความดันเลือด และอุณหภูมิทางทวารหนัก ถูกวัดทุก 5 นาที จนกระทั่งมีการตอบสนองโดยการชักขากลับ การกระตุ้นความเจ็บปวดทางกายภาพและทางไฟฟ้าถูกนำมาใช้เพื่อวัดระดับความลึกของการวางยาสลบ จนมีการตอบสนองต่อการต่อการกระตุ้นความเจ็บปวด จึงมีการให้อะทิพามีโซน 1 มิลลิกรัมต่อกิโลกรัม ใต้ชั้นผิวหนัง ในกระต่ายทุกตัว สังเกตการตอบสนองของกระต่ายโดยการบันทึกวิดีโอ โดยผู้ประเมินที่ไม่ทราบกลุ่มยา ทำการประเมินผลของยาต่อปฏิกิริยาการนำสลบและการฟื้นจากการสลบของกระต่ายทั้งหมดจากบันทึกวิดีโอ ระยะเวลาการนำสลบและระยะเวลาในการวางยามีความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน และกลุ่มยาเคตามีนร่วมกับยาไซลาซีน *(p<0.05)* ค่าเฉลี่ยของเวลาในการนำสลบและระยะเวลาในการสลบ คือ 6.6 และ 110.9 นาทีตามลำดับในกลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน และ 19.8 และ 17.8 นาทีตามลำดับในกลุ่มยาเคตามีนร่วมกับยาไซลาซีน ระยะเวลาในการฟื้นจากยาสลบหลังจากได้รับยาอะทิพามีโซน ไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างกลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน และกลุ่มยาเคตามีนร่วมกับยาไซลาซีน การเปรียบเทียบระหว่างกลุ่มแสดงให้เห็นถึงความแตกต่างอย่างมีนัยสำคัญของ อัตราการเต้นของหัวใจที่นาทีที่ 10 อัตราชีพจรที่นาทีที่ 10 อุณหภูมิทางทวารหนักที่นาทีที่ 25 ความดันเลือดที่นาทีที่ 25 และ 30 หลังจากสูญเสียการทรงตัว *(p<0.05)* ส่วนการเปรียบเทียบภายในกลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน และกลุ่มยาเคตามีนร่วมกับยาไซลาซีน พบว่าอัตราการเต้นของหัวใจและอัตราการหายใจลดลงอย่างมีนัยสำคัญเมื่อเทียบกับเวลาเริ่มต้น (T=0) อุณหภูมิทางทวารทั้งในกลุ่มยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีน และกลุ่มยาเคตามีนร่วมกับยาไซลาซีน มีค่าลดลงอย่างมีนัยสำคัญ ในขณะที่อัตราการเต้นของชีพจร ความดันเลือด และเปอร์เซ็นออกซิเจนอิ่มตัวในเม็ดเลือดแดง ไม่แตกต่างอย่างมีนัยสำคัญ เมื่อเทียบกับเวลาที่นาทีที่ 5 หลังจากสูญเสียการทรงตัว กล่าวโดยสรุปการใช้ยาเคตามีนร่วมกับยาเด็กซ์เมทดีโตมิดีนสามารถควบคุมความเจ็บปวดได้ดีกว่า ให้ระยะเวลาการสลบที่ยาวนานกว่า นำสลบได้รวดเร็วกว่าการใช้ยาเคตามีนร่วมกับยาไซลาซีน อย่างไรก็ตามยาทั้งสองกลุ่มส่งผลกระทบทั้งต่อระบบหมุนเวียนเลือดและการหายใจ ส่งผลให้อัตราการหายใจและอัตราการเต้นของหัวใจลดลงอย่างมีนัยสำคัญ ด้วยเหตุนี้การใช้ยาทั้งสองกลุ่มควรมีการใช้อย่างระมัดระวังในผู้ป่วยที่มีปัญหาเกี่ยวกับระบบทางเดินหายใจ

สาขาวิชา	ศัลยศาสตร์ทางสัตวแพทย์	ลายมือชื่อนิสิต .....
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KEYWORD: analgesia, Dexmedetomidine, ketamine, Rabbit, xylazine

Aomusa Kuaha : A COMPARISON OF KETAMINE COMBINED WITH DEXMEDETOMIDINE AND KETAMINE COMBINED WITH XYLAZINE ON ANALGESIA IN RABBITS. Advisor: Asst. Prof. SUMIT DURONGPHONGTORN, D.V.M., DVSc., D.T.B.V.S. Co-advisor: Asst. Prof. THAWAT LEKDUMRONGSAK, D.V.M., Ph.D.

The effects of ketamine combined with dexmedetomidine and ketamine combined with xylazine on analgesia and cardiopulmonary variables in rabbits were evaluated in eight healthy New Zealand White rabbits. The experiment used a randomized double-crossover design. The rabbits were randomly divided into two groups, Group KD (n=4: 2 males/2 females) receivied 35 mg kg $^{-1}$of ketamine and 0.5 mg kg $^{-1}$of dexmedetomidine SC, while Group KX (n=4: 2 males/2 females) receivied 35 mg kg $^{-1}$of ketamine and 5 mg kg $^{-1}$of xylazine SC. The physiological parameters included HR, RR, PR, SpO_2, SBP and RT were determined every 5 minutes and continuously monitored until the pedal withdrawal reflex returned. Mechanical and electrical stimuli were applied to evaluate the depth of anesthesia. Once the response to the noxious stimuli returned, 1 mg kg $^{-1}$of atipamezole SC was administered in all rabbits. Rabbit reactions were monitored using videorecording. The blinded evaluator assessed the drug effect on anesthesia induction and the recovery reaction of all rabbits from videos recorded. There was significant difference in the induction time and the duration of anesthesia between KD and KX groups ($p<0.05$). The mean induction time and the duration of anesthesia were determined as 6.6 and 110.9 min respectively in the KD group and 19.8 and 17.8 min respectively in the KX group. Atipamezole reversal time was not significantly different between KD and KX. Intergroup comparison exhibited significant difference in terms of HR at the 10th min, PR at the 10th min, RT at the 25th min, SBP at 25th and 30th after loss of the righting reflex ($p<0.05$). As for intragroup comparison, HR and RR in both KD and KX were significantly decreased compared to the baseline (T=0). RT in both KD and KX was significantly decreased while PR, SBP and SpO_2 were not, compared to 5th min after loss of the righting reflex (T=5). In conclusion, KD provided better analgesia, longer anesthesia, more rapid induction time, smoother and less complicated induction and recovery than KX. Both treatments demonstrated cardiopulmonary effects of significant decrease respiratory rate and heart rate. Therefore, both combinations should be used with caution in patients with respiratory depression.

Field of Study:	Veterinary Surgery	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....
		Co-advisor's Signature .....

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INTRODUCTION

1.1 Importance and rationale

Rabbits become common companion pets and are often presented to veterinarians for evaluation and medical treatment. As anesthesia in rabbits is associated with a higher perioperative risk compared with dogs and cats, providing safe and optimized protocol is challenged (Wenger, 2012).

Since the inhalation anesthesia in rabbits has some limitation in use such as difficulty of endotracheal intubation and promptly unavailability of personnel and equipment, the injectable anesthesia is preferred amongst research workers and clinicians because of its ease of administration. Besides, the inhalation anesthesia can cause breath-holding, bradycardia, and hypoxia especially when endotracheal intubation cannot be performed (Kazemi et al., 2002; Murphy et al., 2010). The administration of ketamine combined with xylazine have been one of the recommended and commonly used protocols (Henke et al., 2005). Due to its rapid onset of action with minimal cardiovascular and respiratory depressed effects of ketamine (Bellini et al., 2014) and the use as sedative, analgesia and muscle relaxation of xylazine (Greene and Thurmon, 1988).

However, some disadvantages of using ketamine and xylazine together have been reported. One is that they only can be used for short term painful procedures (Kazemi et al., 2002). In several cases, the combination of ketamine and xylazine can cause hypotension, bradycardia and decrease the respiratory rate (Kazemi et al., 2002; Henke et al., 2005). Moreover, 5.8% death under ketamine and xylazine anesthesia has been reported (Kazemi et al., 2002). These disadvantages are mostly due to less selectivity to alpha 2-adrenorgic receptor agonist of xylazine (alpha 1: alpha 2 ratio of 1:160).

Dexmedetomidine, a selective alpha 2-adrenorgic receptor agonist, has been introduced to veterinary market since 1980's. Due to its high selectivity (alpha 1: alpha 2 ratio of 1:1620), dexmedetomidine provides greater sedative, anxiolytic and longer analgesic effects compared to xylazine (Sinclair, 2003). Moreover, dexmedetomidine can reduce anesthetic requirement and provide hemodynamic stability (Yamamoto et

al., 2007). Despite so many advantages of using dexmedextomidine, it can somehow cause a dose-dependent decrease in heart rate, blood pressure and mild respiratory depression in rabbits (Bellini et al., 2014).

The combination of katamine and xylazine in rabbits have been widely studied. However, to my knowledge, there has not been much data regarding the direct comparison of physiological parameters, the depth of anesthesia and the duration of analgesia associated with the combination of ketamine and xylazine and the combination of ketamine and dexmedetomidine.

1.2 Objective of study

To provide safe with adequate depth injectable anesthesia protocol by comparing the effect of ketamine in combination with dexmedetomidine with the effect of ketamine in combination with xylazine on analgesia, and cardiopulmonary system in rabbits.

1.3 Research question

Does Ketamine combined with dexmedetomidine provide better analgesia and less cardiopulmonary effects than ketamine combined with xylazine in rabbits?

1.4 Hypothesis

Ketamine combined with dexmedetomidine provides better analgesia and less cardiopulmonary effects than ketamine combined with xylazine in rabbits.

1.5 Advantage of study

This study will provide more information about injectable anesthesia using ketamine combined with dexmedetomidine to see whether they provide better analgesia and less cardiopulmonary effects or not. If so, this could be one of the recommended injectable anesthetic protocol in rabbits.

CHAPTER II
LITERATURE REVIEW

2.1 Injectable anesthesia in Rabbits

A balanced anesthesia with a combination of injectable drugs is the preferable protocol for rabbit induction (Wenger, 2012). There are several combinations that can be used to induce the surgical plane of anesthesia such as ketamine 25 to 40 mg/kg combined with either acepromazine 0.25 to 1 mg/kg or diazepam 1-5 mg/kg or xylazine 3-5 mg/kg (Borkowski and Karas, 1999) and fentanyl combined with midazolam or diazepam (Wenger, 2012).

Combinations of injectable anesthesia is used for short diagnostic or surgical procedure. Due to the rapid metabolic rate in rabbit, the injectable doses are usually quite high (Borkowski and Karas, 1999). Intramuscular route can cause discomfort to the rabbits, Hedenqvist et al. (2002) reported that compared to intramuscular injection, subcutaneous route was easy to administer, caused less discomfort and provided similar induction time.

2.2 Injectable anesthetic drugs

2.2.1 Ketamine

Ketamine, a dissociative anesthetic agent, is recommended for anesthesia in rabbits because its rapid onset of action with minimal cardiovascular and respiratory depressant effects (Bellini et al., 2014). Hirota and Lambert (1996) reported that ketamine interacts with N-methyl-D-aspartate (NMDA) receptors, opioid receptors, monoaminergic receptors, muscarinic receptors and voltage-sensitive Ca^{2+} channels. Unlike other drugs, it did not interact with GABA receptors. Clinically, ketamine is administered as a racemate composed of two optical isomers, S (+) and R (-), and there are some pharmacological differences between them. Ketamine is a non-competitive antagonist of the NMDA receptor Ca^{2+}channel pore. It interacts with the phencyclidine binding site leading to significant inhibition of NMDA receptor activity. The NMDA receptor provides excitatory properties which has been implicated in the mechanism of general anesthesia and analgesia. Also, ketamine has been reported to

interact with mu, delta and kappa opioid receptors. It has been shown that ketamine produces 2–3 fold stereoselectivity at mu and kappa receptors but not at delta receptors. Several studies have suggested that ketamine may be an antagonist at mu receptors and an agonist at kappa receptors. These observations suggest that the analgesic effects of ketamine are not mediated via mu opioid receptors in the CNS.

Not only a general anesthesia but ketamine can also be used as a local anesthesia. Ketamine alone is not satisfactory anesthesia for use in rabbits. Ketamine should be given as combination with sedatives or tranquilizers (Kazemi et al., 2002).

2.2.2 Alpha 2-adrenoceptor agonists

Alpha 2 - Adrenoceptor agonists are classified as sedative, muscle-relaxant andanalgesia. They are widely used for chemical restraint and premedication in small and large animals. Even though, alpha 2 - agonists are considered more reliable sedatives, they also have profound effects on other body systems and their use should be limited to healthy patients. The combination of alpha 2-agonist and ketamine is one of the recommended protocol since the muscle relaxant effects of the alpha 2-agonist counteract the rigidity effects of ketamine. Also the alpha 2-agonist provides a smooth recovery and ketamine can moderate some of the unwanted cardiovascular adverse effects of the alpha 2-agonist. Moreover, the popularity of alpha 2-agonists is due to the ability to be reversed by specific alpha 2-receptor antagonists.

Alpha 2-Adrenoceptors are found both centrally and peripherally in pre-and postsynaptic locations. Presynaptic alpha 2-receptors reduce release of the neurotransmitter noradrenaline (norepinephrine) provide a sympatholytic effect. In contrast, activation of postsynaptic alpha 2 receptors triggers a sympathomimetic response similar to the alpha 1-adrenoceptor stimulation. The sedative, analgesic and muscle-relaxant properties of the alpha 2-agonists are mediated at central alpha 2 receptors. Three different alpha 2-adrenoceptor subtypes have been identified: alpha 2a, alpha 2b and alpha 2c. The alpha 2a and 2c-adrenoceptors are found in the central nervous system and appear to be mediating sedation, analgesia and sympatholytic effects. While the alpha 2b-adrenoceptors are found more frequently on vascular smooth muscle and have been shown to mediate vasoconstriction and hypertension (Giovannitti Jr et al., 2015).

In the veterinary use, the alpha 2-agonists are not specific only to the alpha 2-receptors but they also exert some alpha 1 effects. Medetomidine is the most selective, having an alpha2:alpha1 selectivity ratio of 1620:1. While xylazine is much less selective, with alpha2:alpha1 ratios of 160:1. Medetomidine is a racemic mixture of two optical isomers:dexmedetomidine and levomedetomidine. Dexmedetomidine is an active enantiomer and has a potency approximately twice that of the racemic mixture (Sinclair, 2003).

2.2.2.1 Xylazine

Xylazine, (2-(2, 6-dimethyl-phenylamino)-4H-5, 6 dihydro-1, 3 -thiazine), an alpha2 adrenorgic receptor agonist, has less selective effects to the receptors (alpha 1: alpha2 ratio of 1:160). It also effects both vagal and baroreceptor activity via a central mechanism. Xylazine has been introduced to the veterinary market as sedative, analgesic and muscle relaxive agents (Greene and Thurmon, 1988).

Kazemi et al. (2002) reported that ketamine alone is not enough as an injectable anesthesia in rabbits, but the combination of ketamine 35 mg/kg and xylazine 5 mg/kg can be given for short-term painful procedures. However, Henke et al. (2005) reported that ketamine-xylazine provided only short duration of surgical plane, while ketamine and metdetomidine had more prolonged reflex loss in rabbits.

In the study which compared two anesthetic and analgesic protocols for cardiothoracic surgery using rabbits as a model, the combination of ketamine 35 mg/kg, xylazine 5 mg/kg and buprenorphine 0.03 mg/kg or ketamine 35 mg/kg, medetomidine 0.5 mg/kg and buprenorphine 0.03 mg/kg intramuscularly, showed that the metedomidine protocol given to rabbits provided longer duration of anesthesia while the use in dogs and horses had showed no difference in recovery time (Difilippo et al., 2004).

Green et al. (1981) studied the dose of ketamine combined with xylazine and its effect in rabbits. The combination of ketamine dose 25 mg/kg IM and xylazine dose 2 mg/kg IM gave good sedative effect and loss of righting reflex and muscle tone in 10 minutes. The sedative effect last for 2 hours with

responsive to pain throughout. However, the study also reported that rabbits receiving ketamine mixed with xylazine IV at 22 mg/kg and 3 mg/kg respectively achieved surgical anesthesia. The peak effect was attained in 2 minutes and lasted for 35 minutes.

2.2.2.2 Dexmedetomidine

Dexmedetomidine, ((+)-(S)-4-[1-(2, 3-dimethyl-phenyl) ethyl]-1H-imidazole) is a selective alpha-2-adrenorgic receptor agonist which has been introduced to veterinary market since 1980's. Dexmedetomidine is similar to medetomidine. The lack of the pharmacologically inactive enantiomer (levomedetomidine) is making dexmedetomidine 2 times more potent in anesthetic efficacy than medetomidine and 40 times more potent than xylazine (Wellington et al., 2013). For dogs and cats, administration of demedetomidine intramuscularly gives more rapid onset of sedation and better analgesia than with other alpha-2-adrenorgic receptor agonist drugs (Bellini et al., 2014).

Midazolam combined with dexmedetomidine have better sedation quality than combined with ketamine in rabbits. Low doses of demedetomidine has minimal effects to heart rate. The dose of dexmedetomidine used in this experiment was 25 µg/kg given intramuscularly (Bellini et al., 2014).

Yamamoto et al. (2007) reported appropriated dose of demedetomidine used in rabbits which are 100 µg/kg/h as a single anesthesia and 25-50 µg/kg/h as an adjunctive anesthesia. Also the results in this study showed that demedetomidine suppressed myogenic motor evoked potentials in a dose-dependent manner.

The study of a comparison of the respiratory depression associated with dexmedetomidine, propofol, and midazolam in rabbits indicated that dexmedetomidine had less respiratory depression, more hypotension and heart rate reduction.

The combination of dexmedetomidine 0.1 mg/kg, midazolam 2 mg/kg, and butorphanol 0.4 mg/kg administered transnasal to healthy rabbits provided a profound sedation and analgesia suitable for minor surgical procedures.

Trans-nasal administration is a novel route and can be a good option as a chemical restraint (Kim et al., 2004).

A study carried out by González-Gil et al. (2015) evaluated weather various anesthetic mixtures containing dexmedetomidine would alter serum glucocorticoid levels in rabbits which had been using the combination of ketamine dose 35 mg/kg IM and dexmedetomidine dose 0.25 mg/kg IM. The study showed that all rabbits lost 3 monitored reflexes (righting, paw withdrawal and ear pinch) within 10 minutes after injection. The times required for the return of the righting, pedal withdrawal, and ear pinch reflexes were 73.2 ± 19.6, 50.1 ± 19.8, and 51.7 ± 21.7 minutes respectively. The combination of dexmedetomidine and ketamine was associated with increases in both cortisol and corticosterone in rabbits (González-Gil et al., 2015).

Wellington et al. (2013) studied the sedative and physiologic effects of ketamine combined with xylazine (ketamine 100 mg/kg, xylazine 10 mg/kg) and ketamine combined with dexmedetomidine (ketamine 75 mg/kg, dexmedetomidine 0.1 mg/kg) in rats. It is reported that the onset of anesthesia which can be indicated by the loss of the righting, ear pinch, and pedal reflexes was less than 6 minutes after injection in both KX and KD groups. However, the KD group showed more rapid loss of palpebral reflexes than the KX group. The pedal reflex is considered the most sensitive and reliable parameter for surgical plane of anesthesia in rodents. Also, this experiment had reported the result of administration of KD led to bradycardia, respiratory depression, and subsequent hypoxemia, while KX resulted in a shorter period of respiratory depression. Moreover, after 30 minutes of anesthesia, atipamezole dose 1 mg/kg SC was administered and the return of reflexes was recorded. The result indicated that in the KD group, the time to return of the righting, pedal, palpebral, and ear pinch reflexes was significantly longer than that in the KX.

Flecknell (2009) and Longley (2008) has reported the dose of ketamine 35-50 mg/kg combined with dexmedetomidine 0.5 mg/kg was subcutaneously as an injectable anesthesia.

2.2.3 Atipamezole

Atipamezole, (MPV-1248, 4-(2-ethyl-2, 3 dihydro-lH-inden-2-yl)-lH-imidazole) is a lated potent selective and specific alpha-2-adrenogic receptor antagonist that has been shown to be an effective reversal agent for both medetomidine and dexmedetomidine (Aho et al., 1993; Kim et al., 2004). In receptor binding studies in human, Atipamezole has an alpha2/alpha1-selectivity ratio of 8500 compared with 40 for yohimbine. In brains of intact rats, atipamezole increased the release and metabolism of noradrenaline in a dose-dependent manner. Atipamezole rapidly penetrates into the central nervous system causing rapid reversal of the sedative effects. It is a potent antagonist at central alpha2-adrenoceptors (Karhuvaara et al., 1990).

Atipamezole is labeled for intramuscular injection. The pharmacokinetic data shows that the agent has reached its peak plasma concentration in dogs within 15 minutes after intramuscular injection. In addition, atipamezole is rapidly absorbed after subcutaneous injection(Janssen et al., 2017).

For medetomidine, reversal doses of atipamezole is vary between 1and 5 times of medetomidine dose (Wenger, 2012). Kim et al. (2004) investigated that 1 to 2 times higher doses of atipamezole than administered dose of medetomidine is safe and effective for clinical use in rabbits. The same study showed that the period of time to turn righting reflex after administration of atipamezole was dose-dependent. In the group that did not receive atipamezole, this period was about 25-55 minutes.

In the experiment to evaluate the effectiveness of xylazine hydrochloride and its antagonism with atipamezole in Arabian oryx *(Oryx leucoryx),* Ancrenaz (1994) reported that the mean (±SD) reversal time was 87.1 (±43.2) seconds for the recumbent oryx and a resedation period was lasting up to two hours. It occurred between two and five hours after the injection of atipamezole. Compared with the previous studies, it is found that the mean reversal time for the use of yohimbine as a xylaxine reversal agent was longer than that of atipamezole.

In the experiment of Bellini et al. (2014), the excluded rabbits that received dexmedetomidine was administrated atipamezole 1 mg/kg intramuscularly.

2.3 Noxious stimulation techniques

In the study to determine the minimum alveolar concentration (MAC) of an inhalation anesthetic drugs in dogs and rabbits. Various types of noxious stimulation were applied to several anatomic sites. The result of this study indicated that clamping and electrical stimulation were supramaximal stimuli with rabbits, while skin incision was submaximal noxious stimulation. There were no difference between techniques and site of application. Rabbits were clamped at tail, paw of forelimb and hind limb while electrical current were applied to forelimb and hind limb (Valverde et al., 2003).

2.3.1 Mechanical stimulation

Valverde et al. (2004) reported that the mechanical stimulation involved clamping the third and fourth digits of the hind-limb using sponge forceps in order to prevent tissue trauma. The forceps were used to clamp by clicking down to the first notch and applied for a maximum time of 1 minute. The forceps were removed immediately if a purposeful response occurred. Gross movement of the head or limbs, jerking or twisting of the head or running motion of the limbs were considered as a purposeful movement. The same study also reported that both mechanical and electrical stimulus resulted in the same MAC values.

Acevedo-Arcique et al. (2014) applied noxious stimulation by clamping a paw at the fourth digit with 24-cm sponge forceps. The forceps were used to clamped to the first notch for 60 seconds or until purposeful movement was detected.

2.3.2 Electrical stimulation

There was a study of the determinations of minimum alveolar concentration (MAC) in mongrel dogs which used electrical noxious stimulation involving the application of 50 V at 50 Hz for 10 milliseconds using the S48 Stimulator (Astro-Medical, Inc., West Warwick, RI, USA) applied subcutaneously by inserting two 25-G (2.5 cm) needles, 5 cm apart, at the level of the ulna. The sequence of noxious stimulation consists of 2 single stimuli application, followed by 2 continuous stimuli application

for 2 to 3 second, with 5-second intervals, so the animal received four stimuli. Also this method has been reported to give the same result as using the mechanical stimulation (Valverde et al., 2004).

Gianotti et al. (2012) also applied a method involving the electrical noxious stimulation in rabbits which consisted of 50 V at 50 Hz for 10 milliseconds using the S48 Stimulator, applied subcutaneously by inserting two 25-G (0.75cm) needles, 3 cm apart, at the level of the ulna. There was also a study in which the stimulus is applied using a sequence of 2 single stimuli, followed by 2 continuous stimuli applied for 2 to 3 s, with 5-s intervals between all 4 stimuli instead of applying a single stimulus for up to 60 s or less if a positive response was observed.

This variation was chosen since it compared well with clamping techniques and minimized tissue trauma, which may avoid plasticity changed from repeated stimulation.

CHAPTER III
MATERIALS AND METHODS

This study was approved by Chulalongkorn University Laboratory Animal Center Animal Care and Use Committee (Animal Use Protocol No.1973014) and performed at Chulalongkorn University Laboratory Animal Center.

3.1 Animals

Eight (4 males and 4 females) New Zealand White rabbits (*Oryctolagus cuniculus*), weighting 2.2 ± 0.2 kg, were included in the study. The rabbits were supplied from Mahidol University National Laboratory Center. The rabbits were pair housing in standard rabbit cages with rabbits per cage. They were provided commercial rabbit pellets and reverse osmosis water ad libitum. The rabbits were maintained in a controlled environment at 20 ± 2°C , 50 ± 20% humidity and 12:12 h light: dark cycle. The rabbits were acclimated to the facility for 1 week before the experiment. Prior to the study, Physical examination and blood analyses for packed cell volume, total protein, serum glutamic pyruvic transaminase and creatinine were done to confirm their normal health.

3.2 Study design

Prospective randomized double-crossover experimental study

3.3 Study protocols

3.3.1) Experimental design

The experiment used a randomized double-crossover design (Difilippo et al., 2004). Each rabbit received all two combination, KD and KX with 10 days interval (González-Gil et al., 2015). The rabbits were randomly divided into two groups of four animals, Group I (n=4: 2 males/2 females) and Group II (n=4: 2 males/2 females). The combination KX consisted of ketamine (Alfasan®, Diergeneesmiddelen B.V) 35 mg/kg and xylazine (X-Lazine®, LB.S.laboratory) 5 mg/kg, while the KD combination included ketamine 35 mg/kg and dexmedetomidine (Dexdomitor®, Virbac) 0.5 mg/kg (Figure 1).

All drugs were injected subcutaneously. The rabbits received the alternate combination at 10 days after the previous one. Baseline cardiopulmonary parameters (heart rates and respiratory rates) and rectal temperature were taken before each injection.

Figure 1 ketamine (Alfasan®, Diergeneesmiddelen B.V), xylazine (X-Lazine®, LB.S.laboratory) and dexmedetomidine (Dexdomitor®, Virbac).

On the experimental day, each rabbit was weighed and placed into the restrainer where heart rates, respiratory rates, heart sound, lung sound, gut sound, mucous membrane and rectal temperature were recorded.

The time of drug injection was recorded as T0. After the injection of the drug combination, the observer started videorecording of rabbit reaction until lying down. The time at the absence of righting, ear pinch, and paw withdrawal reflexes were observed and the time of loss of the righting, ear pinch, and paw withdrawal reflexes were recorded as T1, T2 and T3 respectively.

The righting reflex was the ability to spontaneously stay on sternal recumbency. The pedal withdrawal reflex was the presence of hind limb withdrawal from clamping the third and fourth digits. This reflex was considered the most sensitive and reliable parameter for surgical plane of anesthesia in rodents (Wellington et al., 2013). Ear pinch reflex was the presence of response to clamping the rim of the ear with Kelly hemostat forceps with protective plastic tubing on the forceps jaws clicked down to the first notch (Kazemi et al., 2002; Difilippo et al., 2004; Henke et al., 2005; Murphy et al., 2010) (Figure 2).

Figure 2 Ear pinch reflex test by clamping the rim of the ear.

The time of positive response to the noxious stimulation was recorded as T4. The duration of anesthesia was the time from the loss until the return of the response to the noxious stimuli (T3-T4).In this experiment, 2 types of the noxious stimuli were applied to evaluate the depth of anesthesia since both mechanical and electrical stimulations were reported to cause similar MAC value in rabbits (Valverde et al., 2003). Mechanical noxious stimuli involved digit clamping at the third and fourth digits of the hind-limb. If there were any purposeful responses such as jerking or twisting motions of the head or running motion of the limbs, the stimulation was stopped and the positive response was recorded (Valverde et al., 2004). Another method, electrical stimulation, was well compared with clamping techniques and minimized tissue trauma (Gianotti et al., 2012). The electrical nerve stimulator (Stimuplex® A, B. Braun Medical Inc.; Bethlehem, PA, USA) was applied during a 20-minute of anesthesia via 24 Ga. x 1 in. (25 mm) insulated needle with 30° bevel and extension set at the level of ulna with frequency of 2 Hz for 0.1 ms. The minimal intensity current (mA) necessary to cause a muscle twitch or sensation were recorded (Hadzic et al., 2004) (Figure 3).

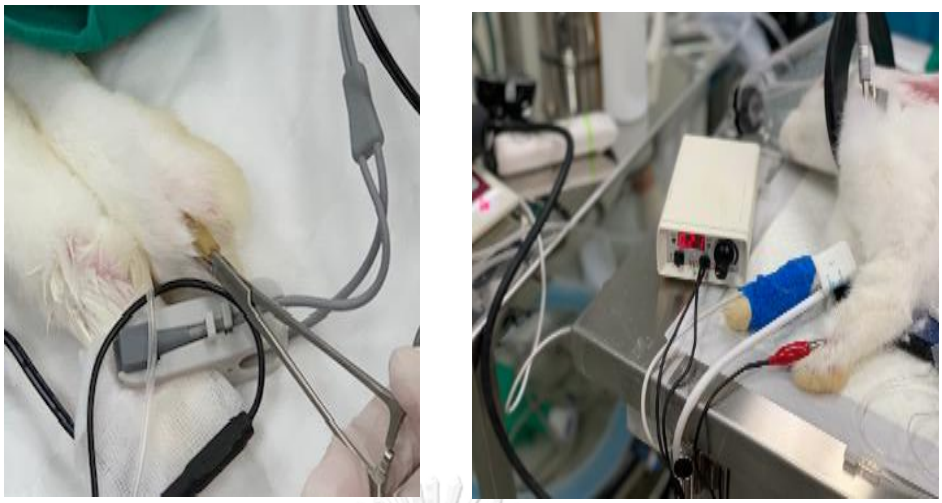

Figure 3 Mechanical noxious stimulation by clamping the third or fourth digit of the hind limb and Electrical noxious stimulation by applying nerve stimulator at the level of ulna.

After the righting reflex was gone, the rabbits were kept quiet and placed on a heating pad, supplemented with 100% oxygen by face mask at 2 L/min, and lubricated their eyes with lubricant (Vidisic gel®, Dr. Mann Pharma). An intravenous catheter was placed in the marginal ear vein, and lactated Ringer's were administered at 10 ml/kg/h. The monitoring equipment were connected and animal physiological parameters were recorded every 5 minutes and continuously monitored until the pedal withdrawal reflex returned. The physiological parameters included heart rates (HR), respiratory

rates (RR), pulse rates (PR), arterial haemoglobin oxygen saturation (SpO2), systolic blood pressure (SBP) and rectal temperature (RT).

HR was measured by electrocardiography from multi-parameter monitor (COMEN C80-V, Shenzhen Comen Medical Instruments Co.,LTD; Guangming, Shenzhen, CHINA). RR was obtained from direct observation of the moving chest over one minute. PR and SpO2 were measured by a pulse oximetry (EDAN iM8 VET series, EDAN CHINA; Shekou, Nanshan Shenzhen, CHINA) (Figure 4). RT was recorded from the temperature probe inserted into the rectum of the animals of the Temperature Monitoring and Homeothermic Control Module (RightTemp, Kent Scientific; Torrington, CT, USA) (Figure 5). SBP was measured with a doppler blood pressure system (Vmed Vet-Dop2, Vmed Technology; Mill Creek, WA, USA) (Figure 6).

Figure 4 HR was measured by Electrocardiography (COMEN C80-V, Shenzhen Comen Medical Instruments Co.,LTD; Guangming, Shenzhen, CHINA). PR and SpO2 were measured by pulse oxymetry (EDAN iM8 VET series, EDAN CHINA; Shekou, Nanshan Shenzhen, CHINA).

Figure 5 Temperature Monitoring and Homeothermic Control Module (RightTemp, Kent Scientific Torrington, CT, USA).

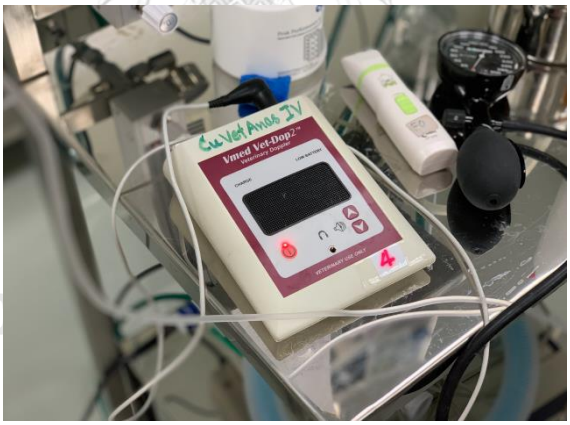

Figure 6 SBP was measured with a doppler blood pressure system (Vmed Vet-Dop2, Vmed Technology; Mill Creek, WA, USA).

Once the response to the noxious stimuli returned, atipamezole (Antisedan®, Virbac) 1 mg/kg was administered subcutaneously (Figure7). The observer again started videorecording of rabbit reaction until getting up. Then the monitoring equipments were disconnected from the animal. However, the righting reflex was continuously monitored until the rabbits return to sternal recumbency (Difilippo et al., 2004). The

time of returning the righting reflex was recorded (T5). Then, the rabbit was moved to a recovery cage.

Figure 7 Atipamezole (Antisedan®,Virbac)

The blinded evaluator assessed the drug effect on anesthesia induction from videos recorded since the drug injection until loss of the righting reflex. Posture of each rabbit was recorded and graded with a scale of 0 (normal) to 4 (loss of the righting reflex) modified from Raekallio et al. (2002) (Table 1.), until the score of 4 were observed. The score of 2 and 3 were recorded as negative signs of induction.

Table 1 Grading scale for the assessment of drug effect on anesthesia.

Score	Spontaneous posture
0	Normal spontaneous posture
1	Sedated but standing/sitting with head up
2	Lying on sternum with head up
3	Lying on sternum with head down
4	Lying on lateral but still responding to stimuli

Modified from (Raekallio et al., 2002)

The same blinded investigator also observed from the videos of the recovery reaction of all rabbits after atipamezole (Antisedan®,Virbac) subcutaneous injection until the right reflex returned.

3.4 Statistical analysis

The data were tested for normality, using the Shapiro-Wilk test. The comparison between the rabbits receiving KD and KX treatments using Mann-Whitney test. The KruskalWallis with the Dunn's multiple comparison test were used to analyze physiological parameters separately within groups receiving KD and KX combinations.

The parametric data were presented as mean ± standard error of the mean (SEM). Non parametric data were present as number and percentage of animals. All statistical analyses were performed using commercial software GraphPad (PRISM® ver. 8, GraphPad, Inc; La Jolla, CA). Statistical significance was defined at a *P*-value of less than 0.05.

CHAPTER IV

RESULTS

4.1 Finding on the anesthetic duration

The time of the absence of righting reflex (T1), ear pinch reflex (T2) and pedal withdrawal reflex (T3) were shown in Table 2. Since the pedal withdrawal reflex was considered as the most sensitive and reliable parameter for surgical plane of anesthesia in rodents (Wellington et al., 2013), the interval time of presence and absence of hind limb withdrawal (T3-T4) was used to measure the duration of anesthesia.

The comparison between KD and KX treatments shown that T1 was not statistically significant differences but T2 and T3 were *($p < 0.05$)*. There was significant difference in the duration of anesthesia (T3-T4) between KD and KX treatments (*$p<0.05$*). Even though loss of the righting reflex time was not difference between the KD and KX treatments, The rabbits receiving KD demonstrated more rapid loss of the ear pinch and paw withdrawal reflexes suggesting that the KD combination did not only provide more rapid induction time but also longer duration of surgical anesthesia than the KX combination.

Table 2 Time of loss of the righting, ear pinch, pedal withdrawal reflexes and duration of loss of pedal withdrawal response of rabbits receiving KD combination and those receiving KX combination. Data presented as minute (mean ± SEM).

Time point	KD	KX
T1 (n=8)	3.0 ±0.2	4.5 ±0.4
T2 (n=8)	4.4 ±0.8*	10.5 ±2.2
T3 (n=8)	6.6 ±1.9*	19.8 ±2.7
T3-T4 (n=8)	110.9 ±5.5***	17.8 ±2.4

(T1- the absence of righting reflex; T2- the absence of ear pinch reflex; T3- the absence of pedal withdrawal reflex; T4- the presence of pedal withdrawal reflex)

Statistically significant difference *(*$P<0.05$*, ** $P<0.01$ and *** $P<0.001$*)* between treatments

In order to evaluate the depth of anesthesia, mechanical and electrical stimulations were applied. During a 20-minute anesthetic equilibration period (T20) the electrical stimulations were applied. While the mechanical stimulation were giving the negative effects, the minimal intensity currents (mA) of the electrical stimulator were not statistically different neither in the rabbit receiving the same combination nor those receiving different combination (Figure 8).

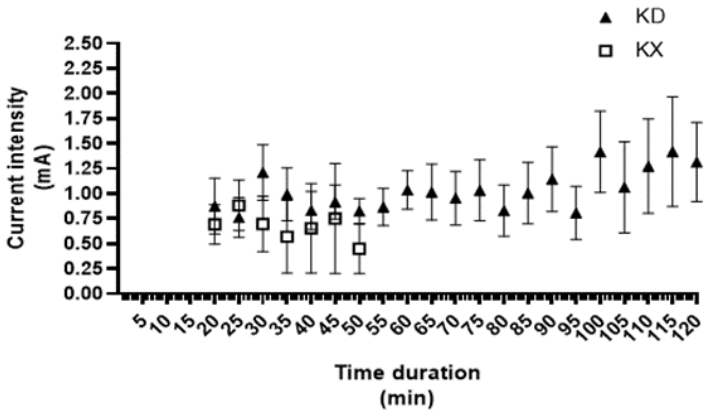

Figure 8 Mean (± SEM) of the intensity current (mA) of the electrical stimulator for eight rabbits receiving KD and KX. T0 = Pre-injection (base line); T5-120 = 5-120 minute after loss of righting reflex; T20 = Minute apply electrical stimuli.

In order to evaluate the drug effect on anesthesia induction from videos, the observation showed number of spontaneous posture score 2 and 3 of rabbits in each group (Figure 9). The score of 2 and 3 were recorded as negative signs of induction.

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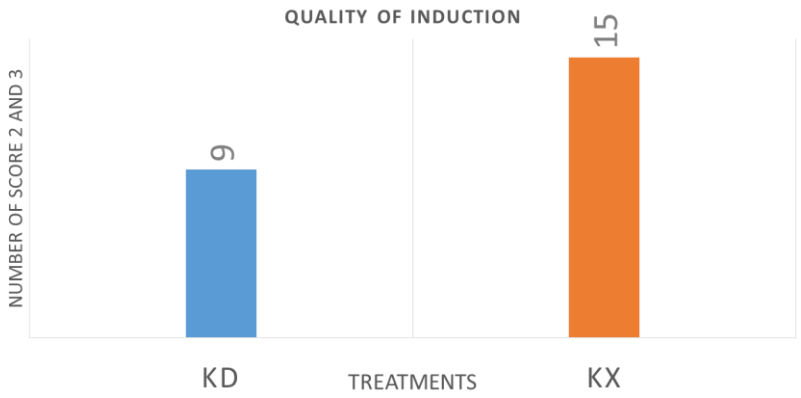


Figure 9 Number of the score 2 and 3 of spontaneous posture score of eight rabbits anesthetized with ketamine 35mg/kg + dexmedetomidine 0.5 mg/kg (KD), ketamine 35 mg/kg + xylazine 5 mg/kg (KX) before loss of the righting reflex (score of 4).

Atipamezole was administrated after response to the noxious stimuli returned. The recovery time was the time from atipamezole injection to return of the righting reflex (T5) which did not differ significantly between KD (6.4 ±0.9 min) and KX groups (6.3± 0.9 min). However, the video observation showed reactions during recovery, including chewing, twitching eyes, nodding head, kicking and rolling (Table 3). Even though, atipamezole reversal time was not different between the rabbits receiving KD and KX combinations, the KD provided smooth and uncomplicated recovery.

Table 3 The recovery reactions after atipamezole injection. Data presented as numbers of rabbits with percentage. (KD- Ketamine and Dexmedetomidine; KX- Ketamine and Xylazine)

Recovery reactions	KD (n=7)	KX (n=7)
Chewing	3 (42.8%)	3 (42.8%)
Twitching eyes	0 (0%)	2 (28.6%)
Nodding head	2 (28.6%)	6 (85.7%)
Kicking and rolling	1 (14.3%)	2 (28.6%)
No sign	3 (42.8%)	1 (14.3%)

4.2 Finding on the cardiopulmonary parameters and rectal temperature

The cardiopulmonary parameters included heart rate (HR, beats per minute), respiratory rate (RR, breaths per minute), pulse rate (PR, beats per minute), arterial haemoglobin oxygen saturation (SpO2, %), systolic blood pressure (SBP, mmHg) and rectal temperature (RT, $^{\circ}$C). All parameters were measured every 5 minutes after loss of the righting reflex and continuously monitored until pedal withdrawal reflex resumed. Before drug injection, only the HR, RR and RT were measured.

Data were presented as mean ±SEM analyzed by using Mann-Whitney test between the KD and KX groups, and the KruskalWallis with the Dunn's multiple comparison test within the KD and KX groups. *P* -value of less than 0.05 was considered significance.

Assessment of HR within group at each time point, there was statistically significant reduction at T25 and T30 in the KX group and at T10, T25, T50, T55,T65 and T105 in the KD group, compared to the baseline (T0 = Preinjection) *(P < 0.05)*. However, the difference of HR between the KD and KX groups was significant at 10 minutes after righting reflex loss (T10) (*P < 0.01*) but insignificant at the other measurement times (T0-T45) *(P > 0.05).* Accordingly, the KD group had significantly lower HR than the KX group at T10 (Figure 10).

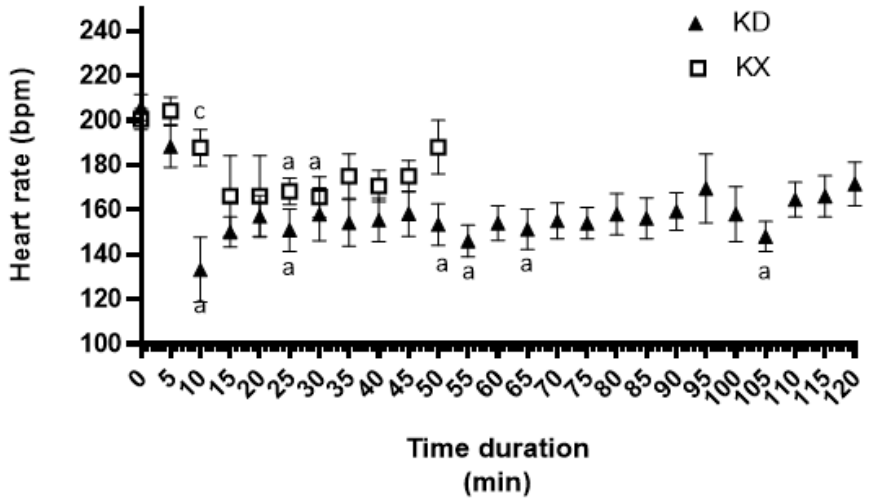


Figure 10 Mean ± SEM of HR at various time point of KD (ketamine and dexmedetomidine) and KX (ketamine and xylazine) groups. T0 = Pre-injection (base line); T5-120 = 5-120 minutes after loss of righting reflex.

[a] is statistically significant difference (P<0.05) from baseline within the same group.

[c] is statistically significant difference (P<0.05) between groups.

There was no difference of RR between the KD and KX groups at all measurement times (T0-T45). Assessment within group at each time point of RR, there was statistically significant reduction at T15-T35 and T45 in the KX group and at T10, T20-T30, T45, T60 and T70 -T100 in the KD group, compared to the baseline *(P < 0.05)* (Figure 11).

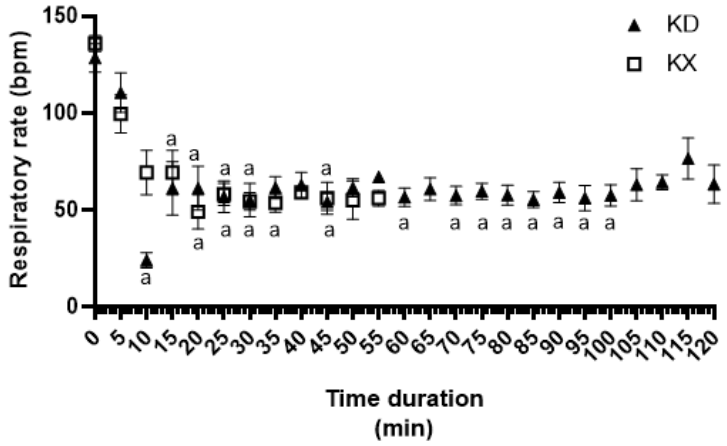


Figure 11 Mean ± SEM RR at various times of KD (ketamine and dexmedetomidine) and KX (ketamine and xylazine) groups. T0 = Pre-injection (base line); T5-120 = 5-120 minutes after loss of righting reflex.

[a] is statistically significant difference (P<0.05) from baseline within the same group.

[c] is statistically significant difference (P<0.05) between groups.

In the present study, temperature monitoring and homeothermic control system was used to maintain normal body temperature during anesthesia. The control

system allowed us to set desired body temperature (38.5°C) to automatically control the warming pad temperature. The difference in RT between the KD and KX groups was significant at 25 minutes after righting reflex loss (T25) and start of the thermostatically control (*P < 0.05*) and insignificant at other time point (T5-T40). Accordingly, at T25, the KD group had significantly higher RT values than the KX group. Assessment of RT at each time point, there was statistically significant reduction at T70 – T120 compared to the RT 5 minutes after loss of righting reflex (T5) and start of the thermostatically control *(P < 0.05)* but no significant difference in any time point compare to the pre injection (T0) in the KD group. While in the KX group, there was statistically significant reduction at T25 compared to T5 and increased at T5- T20 compared to T0. (Figure 12.)

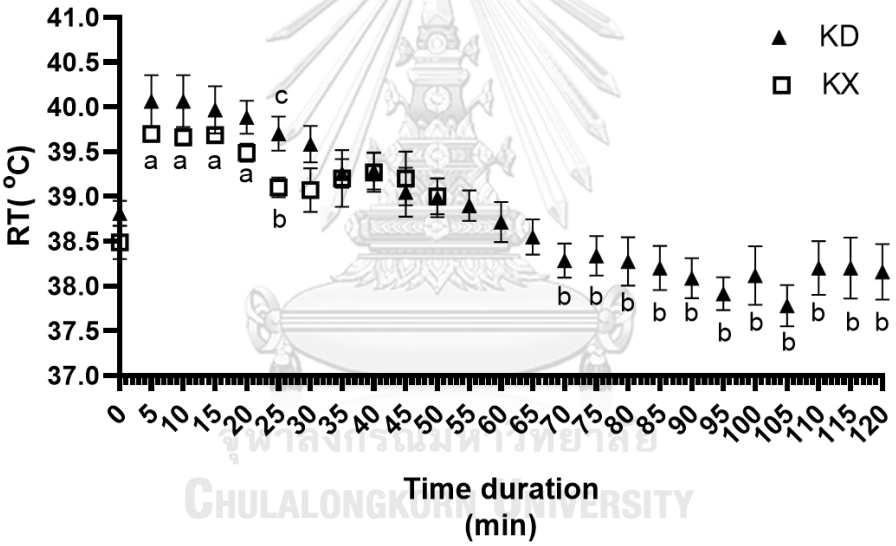


Figure 12 Mean ± SEM of RT at various time points of KD (ketamine and dexmedetomidine) and KX (ketamine and xylazine) groups. T0 = Pre-injection (base line); T5 = 5 minutes after loss of righting reflex and started thermostatically control (base line); T5-120 = 5-120 minutes after loss of righting reflex.

[a] is statistically significant difference (P<0.05) from baseline within the same group.

[b] is statistically significant difference (P<0.05) from T5 within the same group.

[c] is statistically significant difference (P<0.05) between group.

Comparative analyses of SBP between the KD and KX group found significant differences at T25 and T30 (*P < 0.05*) but insignificant difference at other time points (T10-T40). Accordingly, at T25 and T30 with significant difference, the KD group had

significantly lower SBP values than the KX group. In comparison with T10, there was no statistically significant SBP neither in the KD nor the KX groups at any time points (Figure 13).

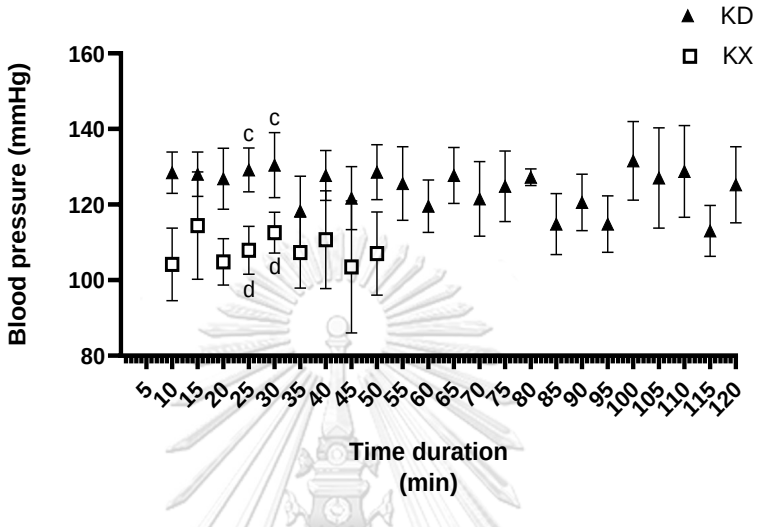


Figure 13 Mean ± SEM of SBP at various time points of KD (ketamine and dexmedetomidine) and KX (ketamine and xylazine) groups. T10 = 10 minutes after loss of righting reflex (base line); T5-120 = 5-120 minutes after loss of righting reflex.

c, d is pair of statistically significant difference (P<0.05) between groups.

The difference in PR between the KD and KX groups was significant at T10 (*P < 0.01)* but insignificant at other measurement time points (T5-T40)*.* Accordingly, at T10 with significant difference, the KD group had significantly lower PR than the KX group. Assessment of PR within group, there was no statistically significant PR neither in the KD nor KX groups at any times points compared to T5. (Figure 14)

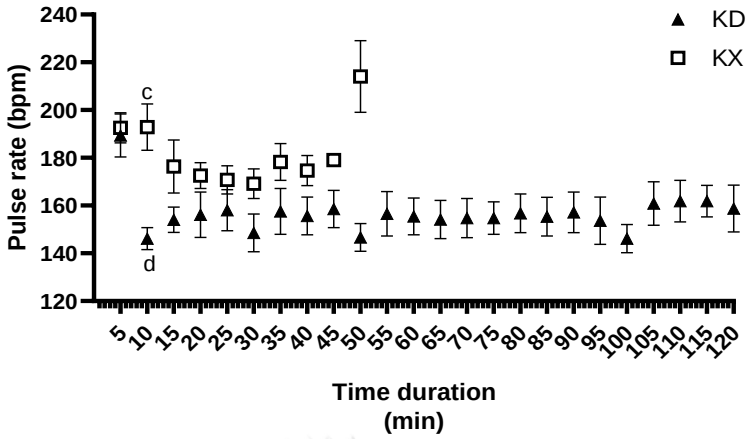
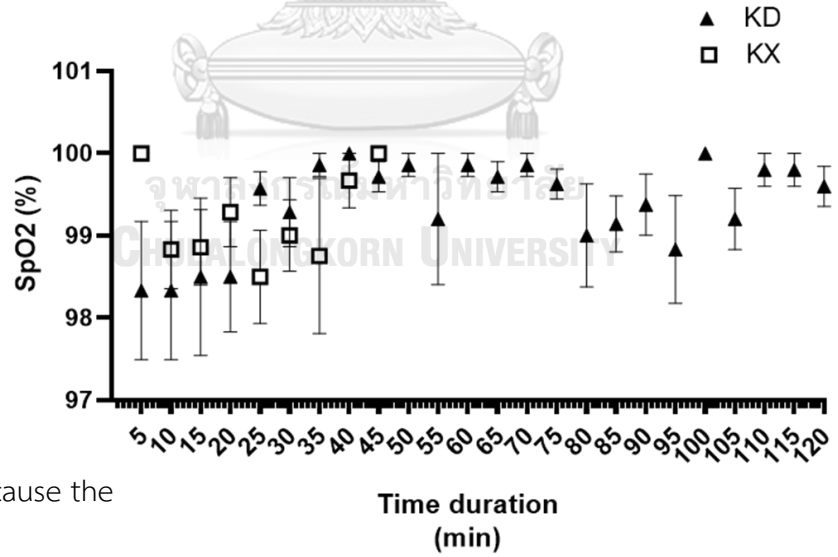


Figure 14 Mean ± SEM of PR at various time points of KD (ketamine and dexmedetomidine) and KX (ketamine and xylazine) groups. T5 = 5 minutes after loss of righting reflex (base line); T5-120 = 5-120 minutes after loss of righting reflex.

c,d is pair of statistically significant difference (P<0.05) between groups.

Also, there was no significant difference in SpO2 at any time points compared to T5 or between treatments (Figure 15).



Because the

Figure 15 Mean ± SEM of SpO2 at various time points of KD (ketamine and dexmedetomidin) and KX (ketamine and xylazine) groups. T5 = 5 minutes after loss of righting reflex (base line); T5-120 = 5-120 minutes after loss of righting reflex.

Because the duration of anesthesia in the KX treatment was shorter than the KD treatment, the comparison of cardiopulmonary parameters in each time event was evaluated; as shown in able 4.

Table 4 Mean ±SEM (n) of HR, RR, RT, PR, SpO2, and SBP of eight rabbits in the KD (ketamine 35mg/kg + dexmedetomidine 0.5 mg/kg) and the KX (ketamine 35mg/kg + xylazine 5 mg/kg).

Parameters		T0	T1	T2	T3	T4
RT (° C)	KD	38.81 ±0.14 (8)	40.07 ±0.29 (6)	40.07 ±0.29 (6)	40 ±0.33 (6)	37.96 ±0.24 (8)[d]
	KX	38.49 ±0.19 (8)	39.7 ±0.09 (6)	39.57 ±0.09 (6)	39.43±0.23 (8)	39 ±0.14 (7)[c]
RR (bpm)	KD	128.38 ±7.45 (8)	110.5 ±10.25 (8)	105.5 ±13.81 (8)	106.13 ±13.32 (8)[c]	69.5 ±8.21 (8)
	KX	135.75 ±4.15 (8)	99.5 ±9.77 (8)	99.2 ±13.11 (5)	56 ±6.93 (6)[d]	53.25 ±3.69 (8)
HR (bpm)	KD	205.75 ±5.90 (8)	188.25 ±9.35 (8)	176.25 ±11.68 (8)	179.25 ±9.86 (8)	162.13 ±5.89 (8)
	KX	200.5 ±4.75 (8)	204.25 ±6.19 (8)	197.17 ±9.45 (6)	177.38 ±9.63 (8)	172.63 ±6.30 (8)
PR (bpm)	KD	ND	189.33 ±8.98 (6)	177.17 ±9.95 (6)	178.83 ±8.89 (6)	163.13 ±6.52 (8)
	KX	ND	192.5 ±6.38 (4)	191.8 ±7.07 (5)	178.5 ±8.44 (8)	177.43 ±10.32 (7)
SpO2 (%)	KD	ND	98.33 ±0.84 (6)	98.33 ±0.84 (6)	98.33 ±0.84 (6)	99.38 ±0.38 (8)
	KX	ND	100 (4)	99.33 ±0.49 (6)	99 ±0.38 (8)	99.14 ±0.40 (7)
SBP (mmHg)	KD	ND	ND	ND	ND	120.57 ±8.66 (7)
	KX	ND	ND	ND	ND	105.14 ±5.52 (7)

T0- Pre-injection; T1 at the absence of righting reflex; T2 at the absence of ear pinch reflex; T3 at the absence of pedal withdrawal reflex; T4 at the presence of pedal withdrawal reflex.

c,d is pair of statistically significant difference ($P<0.05$) between groups.

CHAPTER V
DISCUSSION AND CONCLUSION

The study on different injectable anesthesia protocols has been used in order to provide safe anesthesia with adequate depth and duration in rabbits. The injectable anesthesia is preferred amongst research workers and clinicians because of its ease of administration. The administration of ketamine combined with xylazine, an alpha 2-adrenoceptor agonist, is one of the recommended and commonly used protocols (Henke et al., 2005). Dexmedetomidine, another alpha 2-adrenoceptor agonist, provides greater sedation, anxiolysis and longer analgesic effects compared to xylazine (Yamamoto et al., 2007). An advantage of using ketamine combined with alpha 2-adrenoceptor agonist is the availability of the antagonist, atipamezole (Murphy et al., 2010). Hedenqvist et al. (2002) reported that subcutaneous route was easier to administer, caused less discomfort but provided similar induction time, compared to intramuscular injection.

The present study applied single subcutaneous injection of the KD combination of dexmedetomidine 0.5 mg/kg and ketamine 35 mg/kg (KD), recommended by Flecknell (2009) and Longley (2008), and the combination of xylazine 5 mg/kg and ketamine 35 mg/kg (KX), recommended by Kazemi et al. (2002) and Difilippo et al. (2004). Both combinations were recommended for general anesthesia in rabbits.

Several literatures defined the onset of anesthesia when the righting, ear-pinch and pedal withdrawal reflexes are lost (Kazemi et al., 2002; Difilippo et al., 2004; Henke et al., 2005). Even though loss of the righting reflex time was not difference between the KD and KX treatments, The rabbits receiving KD demonstrated more rapid loss of the ear pinch and paw withdrawal reflexes. In this study, the induction time was 6.6 ±1.9 minutes in the KD group, and 19.8 ±2.7 minutes in the KX group, suggesting that the KD combination provided more rapid induction time than the KX combination. From the experiment of Henke et al. (2005) showed that the combination of ketamine 35 mg/kg and medetomidine 0.25 mg/kg IM provided more rapid loss of the right reflex, ear-pinch reflex and pedal withdrawal reflex than the combination of ketamine 50 mg/kg and xylazine 4 mg/kg IM. The induction time was determined as 5.4 ±4.1 minutes in the KM group and 13.3 ± 9.4 minutes in the KX group.

In the present study, not only the times of loss and return of reflexes were evaluated but animal signs during anesthesia induction was also assessed from videos recording rabbit posture after KD and KX administrations. The rabbit receiving KD showed numbers of negative induction sign (spontaneous posture score 2 and 3) less than the KX. This findings were difference from the study of the sedative effects of ketamine (30 mg/kg) in combination with dexmedetomidine (0.025 mg/kg) and with xylazine (3 mg/kg) which found similar sedative effects in rabbits for up to 20 minutes (Cardoso et al., 2020), since the dose of dexmedetomidine in our study was much higher (0.5 mg/kg).

The recovery is defined when the righting reflex returns after atipamezole injection (Difilippo et al., 2004). Recovery time was the atipamezole reversal time which was not different in both KD and KX. However to assess the quality of recovery, the observed recovery reactions including chewing, twitching eyes, nodding head, kicking and rolling. Rabbits received KX combination showed more reactions than those receiving KD.

In the study by Difilippo et al. (2004) reported the duration to return the righting reflex without receiving atipamezole. The xylazine (5 mg/kg) and ketamine (35 mg/kg)

combination provided 127 ±8 minutes while the medetomidine (0.5 mg/kg) and ketamine (35 mg/kg) combination provide 166 ±19 minutes. From the experiment on determining the optimal reversal dosage of atipamezole on medetomidine and ketamine combination in rabbits, the safe and effective dose of atipamezole was 1 to 2 times of the administered dose of medetomidine (Kim et al., 2004). Since the data on the reversal of dexmedetomidine with atipamezole in rabbits is lacked, the dose of atipamezole selected in this present study was applied from the study in medetomidine. The comparative study on the effects of atipamezole (1 mg/kg IP) and yohimbine (1.5 mg/kg IP) in mice receiving xylazine (10 mg/kg IP) and ketamine (80 mg/kg IP) reported that time to return of the righting reflex in mice receiving atipamezole (10.3 minutes) shorter than in mice reciving yohimbine (21.3 minutes). Also reported, atipamezole is more effective than yohimbine in order to reverse the effect of xylazine (Janssen et al., 2017). Atipamezole was effective to reverse both xylazine and dexmedetomidine and the dose should be minimized for KX in order to avoid anxiogenic effects. The limitation of this study was that there was only one blinded observer assessing both posture scores and recovery reactions.

This study used the pedal withdrawal reflex for evaluating depth of anesthesia to measure the duration of anesthesia (Bienert et al., 2014). The duration of anesthesia in this study was 110.9 ±5.5 minutes in the KD group and 17.8 ±2.4 minutes in the KX group. Bienert et al. (2014) reported that, reflex loss and return times varied based on the dose of dexmedetomidine, and high doses provided longer return times of the pedal withdrawal reflex. In the study where only dexmedetomidine was administered (0.25 mg /kg, intravenous), the duration of loss and return of the pedal withdrawal reflex was determined as 79.25 minutes which was shorter in comparison to our findings. This difference was considered to have been caused by the combined use of ketamine with dexmedetomidine. KİRAZOĞLU et al. (2020) reported that, the combination of ketamine (30 mg/kg) and dexmedetomidine (0.025 mg/kg) IM. Their KD combination in this study using dexmedetomidine of 20 time lower dose rate, provided similar duration of anesthesia (100.5 minutes). Since there has not been much data regarding the comparison between the combination of ketamine and xylazine and the

combination of ketamine and dexmedetomidine, the dose and route of the given and the effects of KD combination should also be studied continuously.

In the study by Karasu et al. (2018), the xylazine (5 mg/kg) and ketamine (50 mg/kg) combination provided 25.8 ±3.5 minutes duration of anesthesia which slightly longer than our study. According to this result, the longer duration of anesthesia was considered to have been associated with the lower dose of ketamine usage (35 mg/kg) in our study. In conclusion, the KD combination provided longer duration of surgical anesthesia than the KX combination.

In the present study, surgery was not performed in the present study. The noxious stimulation applied to evaluate the depth of anesthesia was the mechanical noxious stimuli which involved digit clamping at the third and fourth digits of the hind-limb. Loss of the response to the noxious stimulation was considered the surgical plane of anesthesia. In addition, electrical stimulation was applied at the ulna with the frequency of 2 Hz for 0.1 ms during a 20-minute anesthetic equilibration period. The results showed that during surgical plane of anesthesia, the minimal intensity currents (mA) necessary to cause muscle twitch was not statistically different neither in the rabbit receiving the same combination nor those receiving different combinations. The rabbits receiving KD combination tended to require a higher intensity currents to induce the response which was consistent with our assumption that KD combination provides greater pain tolerance than KX. According to Hadzic et al. (2004) study, a short duration current (≤0.1ms) should be used for nerve stimulation in local anesthesia to stimulate motor response without sensory components. In conclusion, electrical stimulations with frequency of 2 Hz for 0.1 ms might not be suitable for evaluating the depth of anesthesia. However, the limitation of this study were that Stimuplex® A was the only electrical stimulator we had and in the lack of information after the response to mechanical noxious stimuli returned.

In the present study, HR decreased in both KD and KX groups compared with pre-injection HR (baseline) similar to other reports (Cardoso et al., 2020; KİRAZOĞLU et al., 2020). At 25-30 minutes after reflex loss, HR in both groups significantly decreased compared to the baseline. At 10 minutes after loss of the pedal withdrawal, HR in the

KD group was significantly lower than the KX group. However, the KD group had lower HR than the KX group, unlike the findings of KİRAZOĞLU et al. (2020) and Cardoso et al. (2020). Both studies used comparable doses of ketamine (30 mg/kg), dexmedetomidine (0.025 mg/kg) and xylazine (4 and 3 mg/kg, respectively) and found higher HR after KD than KX. The lower HR in KD than KX in our study was due to the higher dose of dexmedetomedine (0.5 mg/kg) supporting Ren et al. (2018) stating that dexmedetomidine decreased heart rate of rabbits in a dose-dependent manner. However, HR in both groups were within the normal limit(Borkowski and Karas, 1999). There were no arrhythmia found in both KD and KX groups.

Assessment of PR within group, there was no statistically significant PR neither in the KD nor KX groups at any times points compared to PR at 5 minutes after loss of righting reflex (T5). Similar to HR, at 10 minutes after loss of the pedal withdrawal, PR in the KD group was significantly lower than the KX group. There was no significant difference between HR and PR.

In the KD group, RR decreased from 10 to100 minutes, while RR in the KX group decreased from 15 to 45 minutes after the righting reflex loss compared with the pre-injection RR. There was no significant difference in RR between KD and KX groups. However, RR in both groups were within the normal limit (Borkowski and Karas, 1999), only at 10 minutes after the righting reflex loss, RR in KD group dropped below normal limit. The mean RR at 10^{th} was 24 ±4 minutes. The result agreed with, the study on the sedation effects of ketamine in combination with dexmedetomidine and xylazine, which reported that RR dropped significantly from 10 minute after injection through 60 minutes of observation time (Cardoso et al., 2020). Moreover, KİRAZOĞLU et al. (2020) reported that RR dropped in the first 15 minutes after KD and the first 30 minutes after KX before increasing in both groups. The similarity of the latter two experiments was the doses of the combination of KD and KX which were lower than we used in our study. The difference was that Cardoso et al. (2020) observed only sedation score while KİRAZOĞLU et al. (2020) tested the reflexes test that could be more painful.

In the present study, temperature monitoring and homeothermic control system was used to maintain normal body temperature during anesthesia. The control system allowed us to set desired body temperature (38.5°C) to automatically control

the warming pad temperature. The difference in rectal temperature between the KD and KX groups was significant only at 25 minutes after righting reflex loss (T25) and start of the thermostatically control ($P < 0.05$), and insignificant at other times (T5-T40). KD group had significantly higher RT than the KX group. For intragroup comparison, there were significant reduction at T25 of the KX group and T70-T120 of the KD group compared to RT at 5 minutes after loss of righting reflex (T5) and start of thermostatically control. Similarity, Karasu et al. (2018) reported that the body temperature of rabbits administered with ketamine (50 mg/kg) and xylazine (5 mg/kg) dropped at 30 minutes after injection, While Cardoso et al. (2020) found the rectal temperature of rabbits administered with ketamine (30 mg/kg) and dexmedetomidine (5 mg/kg) decreased at 60 minutes after injection. The difference of RT at the presence of pedal withdrawal reflex (T4) in both groups was significant, RT in the KD group was much lower than in the KX group. Bellini et al. (2014) reported that body temperature of rabbits decreased rapidly under sedation. In the group received dexmedetomidine, temperature was lower because more time had elapsed and less muscle activity might contribute to the temperature loss during the procedure. Although, RT of the KD group continued to drop over time, RT at T120 (ended point of anesthesia in KD) was not significant different from RT at T0, same as the RT of KX group dropped after 20 minutes of righting reflex lost, compared to RT at T0, RT at T45 (ended point of anesthesia in KX) was also not significant different from RT at T0. However, RT of the KD group was increased for the first 10 minutes and the RT of KX group was significantly increased for the first 20 minutes compared to RT at T0. In general, reduction in temperature with alpha 2-agonist can be attributed to C.N.S depression, in combination with a reduction in muscle activity. Moreover, alpha 2-agonist may allow for better maintenance of body temperature due to the peripheral vasoconstriction and central redistribution of blood, with a consequent reduction in cutaneous heat losses, in contrast to the consistent reductions in body temperature reported with the use of other anesthetic agents that induce vasodilatation (Talukdar et al., 2016).

In the present study, SBP measurement could be started at 10 minutes after loss of the righting reflex (T10). The rabbits receiving KX combination had significant lower SBP than those receiving KD combination at T25 and T30. All rabbits receiving

KD or KX treatments had no hypotension, which was in consistent with the result of the study on hemodynamic response to dexmedetomidine (Bienert et al., 2014). The result showed that higher doses of dexmedetomidine had less effect of decreasing blood pressure. Gallego (2017) reported normal range of systolic blood pressure as 75-134 mmHg. Cardoso et al. (2020) reported that moderated hypoxia was observed in the animal treated with both KD and KX combinations without oxygen supplementation. In the present study, all rabbits supplemented with 100% oxygen, there were no changes in SpO2 in rabbits receiving neither KD nor KX combinations.

In conclusion, the combination of ketamine (35 mg/kg SC) and dexmedetomidine (0.5 mg/kg SC) provided more rapid induction time, better analgesia and longer anesthesia than the combination of ketamine (35 mg/kg SC) and xylazine (5 mg/kg SC). The KD combination also provided smoother and less complicated induction and recovery than the KX. Ketamine (35 mg/kg) combined with dexmedetomidine (0.5 mg/kg) SC is recommended for surgical time less than 110 minutes. Even though, both treatments had similar cardiopulmonary effects of significant decrease respiratory rate and heart rate, there were still within the normal limit and no arrhythmia was found. Therefore, both combinations should be used with caution in patients with respiratory problems. All rabbits receiving KD and KX combinations had no hypotension and change in SpO2, suggested that 100% oxygen Supplement should be provided.

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APPENDIX

Signalment and physical examination

Drugs	Rabbits	Sex	Weight(kg)	Temp (F)	HR	RR	HS	LS	GS	MM
KD	1(R3C1)	Male	2.35	101.6	188	134	normal	normal	normal	pink
	2(R2C2)	Male	2.65	102.4	240	140	normal	normal	normal	pink
	3(R2C3)	Male	2.35	101	198	105	normal	normal	normal	pink
	4(R1C3)	Male	2.3	102.8	216	92	normal	normal	normal	pink
	5(R1C2)	Female	2.2	102.4	200	132	normal	normal	normal	pink
	6(R1C2)	Female	2.25	102.2	212	136	normal	normal	normal	pink
	7(R1C1)	Female	2	102	200	128	normal	normal	normal	pink
	8(R1C1)	Female	1.75	100.8	192	160	normal	normal	normal	pink
KX	1(R3C1)	Male	2.3	100	200	120	normal	normal	normal	pink
	2(R2C2)	Male	2.55	100.8	212	132	normal	normal	normal	pink
	3(R2C3)	Male	2.4	102	208	152	normal	normal	normal	pink
	4(R1C3)	Male	2.35	101.6	200	128	normal	normal	normal	pink
	5(R1C2)	Female	2	101.8	180	152	normal	normal	normal	pink
	6(R1C2)	Female	2.1	99.8	184	126	normal	normal	normal	pink
	7(R1C1)	Female	2.1	102	200	140	normal	normal	normal	pink
	8(R1C1)	Female	1.8	102.4	220	136	normal	normal	normal	pink

Laboratory result

Rabbits No.	1(R3C1)	2(R2C2)	3(R2C3)	4(R1C3)	5(R1C2)	6(R1C2)	7(R1C1)	8(R1C1)
RBC (X106)	7.9	6.91	7.86	7.45	6.08	6.43	6.12	6.71
Hb (g/dl)	16.5	14.1	15.9	13.9	12.5	13.5	12.7	13.6
HCT (%)	51.4	44.7	50.8	44.8	40.7	44.9	42.2	45.4
Blood morphology	Aniso1+	Aniso1+	Aniso1+	Aniso1+	Aniso1+	Aniso1+	Aniso1+	Aniso1+
ALT (Units)	56	83	39	44	49	60	58	41
WBC (per ul)	4080	4770	3950	3950	4220	3520	4000	4760
Neutrophils (%)	26.5	26.3	17.6	16.7	28.3	23	26.9	17.8
Eosinophils (%)	2	1.5	1.7	1.8	2.1	1.7	2	1.9
Basophils (%)	8.1	10.1	5.8	8.6	9.7	10.8	9.3	7.4
Lympho cytes (%)	58.3	55.6	70.5	69.1	50.2	54.8	54.5	66.2
Mono cytes (%)	5.1	6.5	4.4	3.8	9.7	9.7	7.3	6.7
PLT (per ul)	310,000	287,000	167,000	244,000	299,000	321,000	309,000	314,000
Cr (mg%)	0.55	0.43	0.46	0.4	0.44	0.47	0.49	0.4
BUN (mg%)	6.9	6.4	23.9	21.2	20.4	19	25.4	23.2
TP (g%)	8.9	8.3	6.4	5.8	5	6.1	5.5	5.6

Reflex time

Drugs	Rabbits No.	T1 (min)	T2 (min)	T3 (min)	T0-T4 (min)	T3-T4 (min)	T5 (min)
KD	1(R3C1)	3	3	3	144	140	6
	2(R2C2)	2	3	4	110	106	8
	3(R2C3)	4	10	20	115	95	11
	4(R1C3)	3	4	4	112	118	3
	5(R1C2)	3	4	4	89	85	5
	6(R1C2)	3	4	5	118	113	6
	7(R1C1)	3	3	5	116	111	8
	8(R1C1)	3	4	4	123	119	4
KX	1(R3C1)	5	22	30	43	13	2
	2(R2C2)	6	6	28	45	17	5
	3(R2C3)	4	10	12	26	14	9
	4(R1C3)	4	5	17	13	30	6
	5(R1C2)	5	8	25	52	27	6
	6(R1C2)	6	20	25	37	12	10
	7(R1C1)	4	8	11	30	19	5
	8(R1C1)	2	5	10	20	10	7

Recovery sign

	video	Chewing	Twitching eye	Notched head	Kick and roll	No sign
KD	2	/		/		
	4	/				
	7	/				
	10					/
	12			/	/	
	13					/
	15					/
KX	1	/	/	/	/	
	3			/	/	
	6	/	/	/		
	8	/		/		
	11					/
	14			/		
	16			/		

Heart rates, respiratory rates and rectal temperature of eight rabbits received KD (ketamine 35mg/kg + dexmedetomidine 0.5 mg/kg) and KX (ketamine 35 mg/kg + xylazine 5 mg/kg) combinations.

Time point (min)	RT (°C)		RR (bpm)		HR (bpm)	
	KD	KX	KD	KX	KD	KX
T0	38.81 ±0.14 (8)	38.49 ±0.19 8)	128.38 ±7.45 (8)	135.75 ±4.15 (8)	205.75 ±5.90 (8)	200.5 ±4.75 (8)
T5	40.07 ±0.29 (6)	39.7 ±0.09 (6)	110.5 ±10.25 (8)	99.5 ±9.77 (8)	188.25 ±9.35 (8)	204.25 ±6.19 (8)
T10	40.07 ±0.29 (6)	39.66 ±0.09 (5)	24 ±4 (2)[a]	69.25 ±11.50 (4)	133.2 ±14.57 (5)[a]	187.83 ±8.10 (6)[c]
T15	39.97 ±0.27 (6)	39.68 ±0.07 (6)	61.2 ±13.78 (5)	69.25 ±11.50 (4)[a]	150 ±6.78 (4)	166.2 ±17.98 (5)
T20	39.89 ±0.18 (7)	39.49 ±0.10 (8)	61.29 ±11.22 (7)[a]	49 ±8.91 (5)[a]	157 ±9.26 (6)	166.2 ±17.98 (5)
T25	39.7 ±0.19 (8)[b]	39.1 ±0.11 (7)[c]	56 ±8.22 (8)[a]	58 ±5.65 (7)[a]	150.88 ± 9.60 (8)[a]	168.33 ±5.88 (6)[a]
T30	39.59 ±0.20 (7)	39.07 ±0.24 (7)	55 ±8.73 (6)[a]	54.43 ±4.48 (7)[a]	158 ±11.95 (6)	165.67 ±9.19 (6)[a]
T35	39.26 ±0.16 (7)	39.2 ±0.32 (4)	61.57 ±5.66 (7)	53.71 ±4.77 (7)[a]	154.14 ±10.36 (7)	175 ±10.12 (4)
T40	39.29 ±0.21 (7)	39.27 ±0.22 (3)	62.86 ±6.70 (7)	59 ±1.91 (4)	155.43 ±9.80 (7)	170.67 ±7.06 (3)
T45	39.05 ±0.27 (6)	39.2 ±0.3 (2)	54.14 ±4.37 (7)[a]	56 ±8.33 (3)[a]	158.33 ±10.11 (6)	175 ±7 (4)
T50	38.99 ±0.22 (7)	39 ±0.2 (2)	61.43 ±4.58 (7)	55 ±9.98 (4)	153.43 ±9.33 (7)[a]	188 ±12 (2)
T55	38.9 ±0.17 (6)		67 ±1.84 (5)	56 ±4 (2)	146 ±7.15 (5)[a]	
T60	38.72 ±0.22 (6)		56.57 ±4.80 (7)[a]		154 ±7.86 (7)	
T65	38.55 ±0.20 (8)		60.83 ±5.90 (6)		151.29 ±9.00 (7)[a]	
T70	38.29 ±0.19 (7)[b]		57.43 ±4.80 (7)[a]		155 ±8.02 (7)	

Time point (min)	RT (°C)		RR (bpm)		HR (bpm)	
	KD	KX	KD	KX	KD	KX
T75	38.34 ±0.22 (8)[b]		59.63 ±4.25 (8)[a]		154 ±7.12 (8)	
T80	38.28 ±0.27 (8)[b]		57.71 ±5.19 (7)[a]		158 ±9.36 (7)	
T90	38.09 ±0.22 (8)[b]		59 ±5.30 (7)[a]		159.25 ±8.41 (8)	
T95	37.92 ±0.19 (6)[b]		56.17±6.51 (6)[a]		169.6 ±15.39 (5)	
T100	38.12 ±0.33 (6)[b]		57.5 ±5.61 (6)[a]		158 ±12.37 (5)	
T105	37.78 ±0.23 (5)[b]		63 ±8.32 (5)		148 ±6.87 (5)[a]	
T110	38.2 ±0.30 (5)[b]		64.33 ±3.88 (6)		164.5 ±7.84 (6)	
T115	38.2 ±0.34 (5)[b]		76.5 ±10.69 (4)		166 ±9.35 (5)	
T120	38.16 ±0.31 (5)[b]		63.33 ±9.94 (6)		171.5 ±9.86 (6)	

Data presented as mean ± SEM (n). T0 = Pre-injection; T5-120 = minutes after loss of righting reflex

[a] is statistically significant difference *(P<0.05)* from baseline within the same group.

[b] is statistically significant difference *(P<0.05)* from T5 within the same group.

[c] is statistically significant difference *(P<0.05)* between groups at the same time point.

Mean ±SEM (n) of pulse rate (PR), arterial haemoglobin oxygen saturation (SpO2) and systolic blood pressure (SBP) of eight rabbits receiving KD (ketamine 35mg/kg + dexmedetomidine 0.5 mg/kg) and KX (ketamine 35 mg/kg + xylazine 5 mg/kg) by the subcutaneous.

Time point (min)	PR (bpm)		SpO2 (%)		sBP (mmHg)	
	KD	KX	KD	KX	KD	KX
T0	ND	ND	ND	ND	ND	ND
T5	189.33 ±8.98 (6)	192.5 ±6.38 (4)	98.33 ±0.84 (6)	100 (4)	ND	ND
T10	146.17 ±4.61 (6)[c]	192.86 ±9.71 (7)[d]	98.33 ±0.84 (6)	98.83 ±0.48 (6)	128.4 ±5.53 (5)	104.17 ±9.60 (6)
T15	154 ±5.34 (4)	176.33 ±11.10 (6)	98.5 ±0.96 (4)	98.86 ±0.46 (7)	128 ±5.89 (4)	114.4 ±14.23 (5)
T20	156.17 ±9.49 (6)	172.57 ±5.41 (7)	98.5 ±0.67 (6)	99.29 ±0.42 (7)	126.8 ±8.11 (5)	104.86 ±6.18 (7)
T25	158 ±8.68 (7)	170.71 ±5.91 (7)	99.57 ±0.20 (7)	98.5 ±0.57 (8)	129.2 ±5.85 (5)[c]	107.88 ±6.38 (8)[d]
T30	148.5 ±7.94 (6)	169.14 ±6.20 (7)	99.29 ±0.42 (7)	99 ±0.44 (7)	130.4 ±8.61 (5)[c]	112.57 ±5.42 (7)[d]
T35	157.57 ±9.64 (7)	178.25 ±7.75 (4)	99.86 ±0.14 (7)	98.75 ±0.95 (4)	118.33 ±9.26 (6)	107.33 ±9.40 (3)
T40	155.63 ±7.96 (8)	174.67 ±6.36 (3)	100 (8)	99.67 ±0.33 (3)	127.67 ±6.60 (6)	110.67 ±12.98 (3)
T45	158.57 ±7.81 (7)	179 ±2 (2)	99.71 ±0.18 (7)	100 (2)	121.67 ±8.37 (6)	103.5 ±17.5 (2)
T50	146.67 ±5.82 (6)	214 ±15 (2)	99..86 ±0.14 (7)		128.57 ±7.29 (7)	107 ±11 (2)
T55	156.5 ±9.40 (6)		99.2 ±0.8 (5)		125.6 ±9.79 (5)	

Time point (min)	PR (bpm)		SpO2 (%)		sBP (mmHg)	
	KD	KX	KD	KX	KD	KX
T60	155.43 ±7.75 (7)		99.86 ±0.14 (7)		119.6 ±6.94 (5)	
T70	154.71 ±8.27 (7)		99.86 ±0.14 (7)		121.5 ±9.89 (6)	
T75	154.75 ±6.80 (8)		99.63 ±0.18 (8)		124.86 ±9.39 (7)	
T80	156.75 ±8.14 (8)		99 ±0.63 (8)		127.2 ±2.24 (5)	
T85	155.38 ±8.08 (8)		99.14 ±0.34 (7)		114.83 ±8.03 (6)	
T90	157.13 ±8.51 (8)		99.38 ±0.38 (8)		120.57 ±7.49 (7)	
T95	153.67 ±9.87 (6)		98.83 ±0.65 (6)		114.8 ±7.50 (5)	
T100	146.2 ±5.94 (5)		100 (6)		131.6 ±10.40 (5)	
T105	160.83 ±9.09 (6)		99.2 ±0.37 (5)		127 ±13.30 (4)	
T110	161.8 ±8.70 (5)		99.8 ±0.2 (5)		128.75 ±12.15 (4)	
T115	161.83 ±6.62 (6)		99.8 ±0.2 (5)		113 ±6.76 (4)	
T120	158.75 ±9.82 (4)		99.6 ±0.24 (5)		125.2 ±10.13 (5)	

(ND- not determine) T0- Pre-injection; T5-120 = 5-120 minutes after the loss of righting reflex).

[c,d] is pair of statistically significant difference (P<0.05) between groups.

Mean (± SEM) (n) and Median with range of the intensity current (mA) of the electrical stimulator of eight rabbits receiving KD (ketamine 35mg/kg + dexmedetomidine 0.5 mg/kg) and KX (ketamine 35 mg/kg + xylazine 5 mg/kg) by the subcutaneous.

Time point (min)	Current (mA)	
	KX	KD
T0	ND	ND
	ND	ND
T5	ND	ND
	ND	ND
T10	ND	ND
	ND	ND
T15	ND	ND
	ND	ND
T20	0.86 ±0.3(4)	0.69 ±0.2 (6)
	0.79 (0.29-1.63)	0.63 (0.21-1.22)
T25	0.76 ±0.2(7)	0.88 ±0.25(7)
	0.6 (0.27-1.75)	0.9 (0.21-1.85)
T30	1.21 ±0.28(6)	0.7 ±0.28(5)
	1.2 (0.47-1.91)	0.3 (0.21-1.6)
T35	0.99 ±0.27(7)	0.57 ±0.36 (3)
	0.87 (0.31-2)	0.21 (0.2-1.3)
T40	0.83 ±0.19(8)	0.65 ±0.45(3)
	0.78 (0.4-1.9)	0.21 (0.2-1.55)
T45	0.9 ±0.17(8)	0.75 ±0.55(2)
	0.8 (0.41-1.9)	0.75 (0.2-1.3)
T50	0.82 ±0.12(7)	0.45 ±0.25(2)
	0.78 (0.36-1.28)	0.45 (0.2-0.7)
T55	0.86 ± 0.19 (4)	ND
	0.97 (0.34-1.17)	ND
T60	1.04 ±0.19(7)	ND
	0.93 (0.4-1.7)	ND
T65	1.02 ±0.28(6)	ND
	0.97 (0.21-1.93)	ND
T70	0.82 ±0.12(7)	ND
	0.83 (0.21-1.75)	ND
T75	1.03 ±0.3(7)	ND
	1.06 (0.2-2.36)	ND
T80	0.83 ±0.26(6)	ND
	0.8 (0.2-1.75)	ND
T85	1.03 ±0.3(7)	ND
	0.92 (0.2-2.36)	ND
T90	1.15 ±0.32(7)	ND
	1.22 (0.2-2.37)	ND
T95	0.80 ±0.27(5)	ND
	0.56 (0.21-1.63)	ND
T100	1.41 ±0.41(6)	ND
	1.35 (0.21-2.62)	ND
T105	1.06 ±0.46(5)	ND
	0.6 (0.21-2.6)	ND
T110	1.27 ±0.47(6)	ND
	0.9 0.21-2.8)	ND
T115	1.42 ±0.55(4)	ND
	1.34 (0.21-2.8)	ND
T120	1.32 ±0.39(5)	ND
	1.14 (0.21-2.6)	ND

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