MEKELLE UNIVERSITY

COLLEGE OF VETERINARY SCIENCES

DEPARTMENT OF VETERINARY SURGERY AND ANATOMY

EFFECT OF MIDAZOLAM AND ACEPROMAZINE WITH KETAMINE COMBINATION ON CLINICO-PHYSIOLOGICAL AND HEMATO-BIOCHEMICAL PARAMETERS IN SHEEP IN MEKELLE, TIGRAY, ETHIOPIA

By

HAILAY KAHSAY

(ID No.: CVM/PR010/12)

A Thesis Submitted to the College of Veterinary Sciences, Mekelle University, In Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Surgery and Diagnostic Imaging

November 2023

Mekelle, Ethiopia

‘DECLARATION`

I declare that this thesis presents the work carried out by myself and does not incorporate without the acknowledgment of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented, including jointly authored publications, are clearly acknowledged.

Name of the Candidate: Hailay Kahsay Signature: _____

EFFECT OF MIDAZOLAM AND ACEPROMAZINE WITH KETAMINE COMBINATION ON CLINICO-PHYSIOLOGICAL AND HEMATO-BIOCHEMICAL PARAMETERS IN SHEEP IN MEKELLE, TIGRAY, ETHIOPIA

By

HAILAY KAHSAY

(ID No.: CVM/PR010/12)

BOARD OF EXTERNAL EXAMINERS **Signature**

1._____ _____

2._____ _____

3._____ _____

Advisor:

Guesh Negash (DVM, MVSc), Associate Professor _____

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the almighty GOD for his mercy.

Next, my gratitude is directed towards my advisor, Dr. Guesh Negash for his ongoing support, encouragement, academic guidance, active persuasion, and supervision. His efforts served as a constant source of inspiration for me throughout the duration of my study and research activities.

I would like to express my sincere gratitude to Dr. Yohannes Hagos for the data analysis.

My sincere gratitude goes to all my family members and friends for helping me finish my studies and for their support in doing so.

TABLE OF CONTENTS

LIST OF TABLES

List of tables Pages

LIST OF ABBREVIATIONS

ACH	Acetylcholine
AK	Acepromazine-Ketamine
BW	Body weight
CSA	Central statistical agency
DLC	Differential Leukocyte Count
EDTA	Ethylene diamine tetra acetic acid
GABA	Gamma-Amino Butyric Acid type A receptors
HBC	Hemoglobin Concentration
IM	Intramuscular
IV	Intravenous
KG	Kilo gram
Mg	Milligram
MK	Midazolam-ketamine
NMDAR	N-Methyl-D-aspartate receptor
$Paco_2$	Partial pressure of carbon dioxide
Pao_2	Partial pressure of oxygen
PCV	Packed Cell Volume
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
SQ	Subcutaneous
TEC	Total erythrocyte count
TLC	Total leukocyte count

ABSTRACT

An experimental study was carried out from July to October 2023 to evaluate the effects of the general anesthetic combinations of Midazolam-Ketamine and Acepromazine-Ketamine on clinico-physiological and hematobiochemical parameters in sheep in Mekelle, Tigray, Ethiopia. Eight sheep were used in this experimental study. The sheep were randomly divided into two groups with four sheep each (two male and two female), of which four sheep were randomly assigned to an induction regimen of Midazolam-Ketamine (Group MK) and the other four sheep were assigned to Acepromazine-Ketamine (Group AK). Physical recording and laboratory analysis were used to collect the data. The collected data included anesthetic indices, physiological, hematobiochemical, and adverse effects of the anesthetic combinations. All recorded data were entered into a Microsoft Excel spreadsheet and analyzed with SPSS version 23.0. A paired t-test was used to compare the clinico-physiological and hematobiochemical measurements. The significant difference in mean values at a 95% confidence interval (CI) was assessed using an independent t-test. To pinpoint the combinations' negative impacts, the Fisher's exact test was used. In this study, Acepromazine-Ketamine combination had a shorter sternal recumbency time (4.17 ± 0.64 min) and induction of anesthesia (7.85 ± 3.73 min). In contrast, the Midazolam-Ketamine combination had a longer induction of anesthesia (15.10 ± 4.28 min) and sternal recumbency time (5.53 ± 0.22 min). The duration of anesthesia (43.3 ± 1.12 min) and recovery period (49.89 ± 5.10 min) were longer in the Midazolam-Ketamine combination, whereas the duration of anesthesia (17.01 ± 6.27 min) and recovery period (33.61±5.92 min) was shorter in the Acepromazine-Ketamine combination. Following administration of the Midazolam-Ketamine combination, the respiratory rate and the heart rate increased significantly. The experiment showed that the combination of Acepromazine-Ketamine produced stable surgical anesthesia for a short duration, whereas the Midazolam-Ketamine combination was the choice of general anesthesia for a longer duration.

Keywords: *Acepromazine; General anesthesia; Ketamine; Midazolam; Sheep*

CHAPTER I

1. INTRODUCTION

1.1. Background

General anesthesia is a regulated, drug-induced intoxication of the central nervous system (CNS) that results in a state of reversible unconsciousness in which the patient is neither aware of nor able to recall unpleasant stimuli. No one anesthetic agent can induce all of the effects of general anesthesia without impairing the function of some crucial organs. Therefore, a multi-drug strategy is used to reduce sympathetic, parasympathetic, and motor reflex activity, as well as to attenuate certain anesthetic state components. Generally speaking, the premedication, induction, maintenance, and recovery phases of the anesthetic regimen should be separated. To achieve adequate hypnosis, myorelaxation, and analgesia throughout each of these phases, the right medicines or a combination of drugs must be employed (Toholj *et al.*, 2014).

Ruminants typically undergo a variety of surgical procedures under a combination of physical restraint, sedation, and local or regional anesthesia. While there are risks associated with anesthesia, small ruminants use a variety of anesthetic medicines to perform anesthesia. Balanced anesthesia is often used to reduce anesthetic problems (Mohiuddin *et al.*, 2018). Combining two or more anesthetic medicines to achieve the desired aspects of general anesthesia while minimizing the negative effects of individual drugs on cardiopulmonary function is a balanced anesthetic approach (Rubio *et al.*, 2022). Ketamine, a dissociative anesthetic that causes a strong analgesic effect, has been used to keep animals unconscious, either by intermittent bolus infusions or continuous intravenous infusions. When used as a sole agent, it increases heart rate and mean arterial pressure, improves cardiovascular functions, and can have negative side effects such as muscle hypertonicity, myoclonus, and seizures (Marland *et al.*, 2013).

In sheep, sedation and general anesthesia are typically straightforward, except for noticeable regurgitation and potentially fatal pulmonary aspiration. The main issues with general anesthesia in ruminants also include salivation, tympany, and regurgitation of

rumen contents. In sheep, anesthesia with a quick induction, easy transition, and shorter recovery time is preferred (Clarke *et al.*, 2013).

1.2. Statement of the problem

General anesthesia is a prerequisite in sheep for surgical treatment, but sheep differ from other animals by specific anatomical and physiological characteristics, for example, low volume of gases, retention of regurgitation of gases causing tympany, relaxation of the esophageal muscles allowing reflux of rumen content, and disturbances in swallowing. Sedation and general anesthesia in sheep are usually uncomplicated except for notable regurgitation with potentially fatal pulmonary aspiration. In addition, salivation, tympany, and regurgitation of rumen contents are the chief difficulties during general anesthesia in ruminants (Ashraf *et al.*, 2019).

Ketamine is a dissociative anesthetic agent and produces profound analgesia; it has been used for the maintenance of anesthesia, either by intermittent bolus infusion or continuous intravenous infusion. However, using ketamine alone has two drawbacks: it provides short-term anesthesia, which is not ideal for lengthy surgical procedures, and inadequate muscle relaxation, where muscle tone is frequently raised. Therefore, it would be excellent to combine ketamine with some chemicals that aid in getting beyond these restrictions (Leece, 2017).

Midazolam, a benzodiazepine with a high margin of safety, interacts with sedative and anticonvulsant effects as well as glycine-mediated inhibitory pathways in the brain and spinal cord to provide anxiolytic and muscle-relaxing effects. Instead of directly reducing neuronal activity, it works indirectly by enhancing an already-existing physiological inhibitory pathway, and it quickly crosses the blood-brain barrier (Marjani *et al*., 2015).

Acepromazine possesses antiemetic, anticonvulsant, antispasmodic, hypotensive, and hypothermic properties, and its administration produces muscle relaxation with no analgesic effect (Tranquilli *et al.*, 2007).

The aforementioned anesthetic drugs cannot provide the necessary effect when used alone. But when combined, they can have a smooth anesthetic effect.

1.3. Objectives

1.3.1. General objective

The objective of this study was to evaluate the effect of anesthetic combinations of Midazolam-Ketamine and Acepromazine-Ketamine in sheep.

1.3.2. Specific objectives

The current investigation was conducted with the following goals in mind:

- To evaluate the physiological parameters immediately before and after administration of the anesthetic combinations.
- To assess the anesthesia indices of the two combinations
- To determine the effect of the biochemical and hematological parameters.
- To record the negative effects of the anesthetic combinations

1.4. Significance of the Study

Every surgical procedure involving animal patients must be performed under anesthesia. Thus, creating a dependable and safe anesthetic procedure for use in clinics and in the field is crucial for managing surgical disease in sheep. As a result, researching how ketamine interacts with midazolam and acepromazine may aid in developing the safest mix for surgical procedures in sheep.

CHAPTER II: LITERATURE REVIEW

2.1. General Anesthesia in Sheep

For some surgical procedures in sheep, general anesthesia is applied. Numerous sedatives, tranquilizers, painkillers, and muscle relaxants are also utilized during surgery on sheep. These anesthetics support sustaining anesthesia depth, enhancing safety, and overcoming animal resistance during examination. The most widely utilized anesthetic drugs used as anesthetic agents in sheep are atropine sulfate, ketamine, xylazine, and diazepam (Mahmud *et al.*, 2014).

The rumen, other abdominal viscera, or the gravid uterus block ventilation when a sheep is in a lateral or dorsal recumbency, which causes hypoxemia and hypercapnia (Dunlop and Hoyt, 1997). Whereas the incorrect placement causes a mismatch in pulmonary ventilation and perfusion, additionally, respiratory discomfort is made worse by lateral or dorsal recumbency, strong sedation, and anesthesia; gas produced by continuous ingesta fermentation accumulates in the rumen and causes tympany. Additionally, the abdominal viscera compress the major abdominal veins and prevent venous blood flow when the animal is in a dorsal recumbent position, which lowers cardiac output, blood pressure, and tissue perfusion. Withholding food and water for adult animals for 12 to 18 hours and 8 to 12 hours, respectively, lessens the intensity of tympany while having little to no impact on the volume of ruminal contents. However, this has adverse consequences for cardiorespiratory performance (Skarda, 1996).

Premedication is allegedly rarely essential and frequently undesirable in sheep due to its potential to promote regurgitation and lengthen recovery. However, premedication makes handling and inducing anesthesia in difficult-to-handle animals safer, significantly lowers the amount of anesthetic needed and, consequently, the incidence and severity of any side effects, offers preventative analgesia, and promotes easy recovery. Premedication appears to have much more benefits than drawbacks in the majority of circumstances. Smaller doses of sedative medications can also be used as premedication. Benzodiazepines do not have analgesic effects; however, they do have modest sedative, muscle relaxant, and anticonvulsant properties (Grimm *et al.*, 2017).

2.2. Midazolam

Midazolam is a benzodiazepine tranquilizer with sedative, anxiolytic (anti-anxiety), and muscle relaxant properties. It is often used in combination with other anesthetic agents to enhance sedation and provide muscle relaxation. Midazolam can help reduce the dose requirements of other anesthetics. Midazolam is a water-soluble imidazobenzodiazepine that differs structurally from other benzodiazepines by the presence of an imidazole ring. In its closed form at physiological pH, this ring imparts increased lipid solubility, facilitating tissue penetration. This characteristic has contributed to its being an extensively studied benzodiazepine for spinal administration. As a single drug administered by intrathecal bolus in humans, midazolam doses up to 2 mg/dl have effectively treated chronic nonmalignant back pain (Serrao *et al.*, 1992) and pain of somatic origin (Goodchild and Noble, 1987). In rodent, dog, and sheep models, intrathecal midazolam alone has shown effects of sensory blockade and antinociception and significant increases in mechanical pain thresholds (Goodchild and Serrao, 1987).

Midazolam is not as frequently utilized in veterinary medicine as drugs like diazepam. It has been reported to produce profound central nervous system depression in dogs when administered in combination with xylazine and butorphanol (Tranquilli *et al.*, 1991) and with ketamine in cats (Chambers and Dobson, 1989). Midazolam has a minimal effect on the cardiopulmonary system in pigs (Smith *et al.*, 1991) and dogs (Vineet and Bharat, 2007). It has been observed that when given to dogs along with xylazine and butorphanol, it causes severe central nervous system depression in the animals (Tranquilli *et al.*, 1991) and with ketamine in cats (Marjani *et al*., 2015). In pigs and dogs, midazolam has very little impact on the cardiopulmonary system (Bustamante and Valverde, 1997). Establishing a sedative dose and studying the effects of midazolam and droperidol on pigs' cardiovascular systems (Simon *et al.*, 2014)

It is most likely that midazolam interacts specifically with the GABA/benzodiazepine receptor complex in sheep to cause anti-nociception (Roberts *et al*., 2000). Additionally, it is utilized in the induction of anesthesia in humans (Michaloudis *et al.*, 1995). In rats, a powerful sedative effect is produced when medetomidine and midazolam are combined;

this is most likely the result of a synergistic interaction between the two drugs (Salonen *et al*., 1992), pigs (Nishimura *et al.*, 1994), and dogs (Hayashi *et al.*, 1994).

2.3. Acepromazine

Acepromazine is a phenothiazine tranquilizer commonly used as a pre-anesthetic agent in sheep. It provides sedative and calming effects, reduces anxiety, and can enhance the effects of other anesthetic agents. Acepromazine is typically administered before the induction of anesthesia to help facilitate a smooth transition. It exerts sedative, anti-arrhythmic, and vasodilator properties, which are considered to explain the protective effect of this drug against perioperative mortality during general anesthesia. It is a strong neuroleptic agent. It is a powerful antagonist of the post-synaptic dopamine receptor D_2 and, to a lesser extent, of the other D_2-like receptors. Additional effects are connected to its notable antagonistic actions on numerous other receptors, such as the ACh, H_1, and α_1 receptors. The liver converts it into its main metabolite, hydroxyethylpromazine sulfoxide, which is then eliminated in the urine. Its ability to have an antiemetic effect is due to its actions at the solitary nucleus (in the medulla oblongata) and the chemoreceptor trigger zone. When deworming with piperazine, acepromazine should not be utilized. It reduces blood pressure (Johansen *et al.*, 2004).

Acepromazine is used in anesthetic cocktails, combined with ketamine or with ketamine and xylazine, to prolong anesthesia and reduce the doses of ketamine required. Dose-dependent reduction of blood pressure results from vasodilatation, apparently mediated by blockade of peripheral α1-adrenoreceptors. Acepromazine maleate produces mild sedation and skeletal muscle relaxation in ruminants. When administered at a dose of 0.02–0.1 mg/kg IV or SQ, acepromazine provides mild tranquilization with minimal respiratory depression (Clifford, 1984).

2.4. Ketamine

Ketamine acts primarily as an antagonist of the N-Methyl-D-aspartate (NMDA) receptor. Ketamine's anesthetic, amnesic, dissociative, and hallucinogenic effects are caused by NMDAR antagonism; however, its hallucinogenic effects may also result from the

activation of kappa-opioid receptors and sigma and ACh receptors. Ketamine is a dissociative anesthetic that provides profound sedation and analgesia. It acts on the central nervous system and induces a trance-like state, dissociating the patient from their environment. Ketamine is frequently combined with other sedatives or induction agents for a balanced anesthesia protocol. Ketamine may have antidepressant benefits when used in smaller dosages, although the exact mechanisms of action are still unknown. By reducing central sensitization in dorsal horn neurons due to NMDAR antagonism, ketamine blocks the transmission of pain signals in the spinal cord. Nitric oxide synthase inhibition reduces the generation of nitric oxide, a neurotransmitter implicated in pain perception, further enhancing analgesia. Evidence suggests that ketamine's effects on the sigma and mu-opioid receptors are quite mild (Clarke *et al*., 2013).

Ketamine has a wide range of effects, including bronchodilation, analgesia, anesthesia, hallucinations, and arterial hypertension. In conjunction with a few sedatives, it is used to induce and maintain general anesthesia. In contrast to other anesthetics, ketamine has a different impact on the respiratory and circulatory systems. It often stimulates the circulatory system when given at anesthetic levels (Adams, 1997).

On the circulatory and respiratory systems, the effects of intravenous ketamine injections in sheep are contrasted with those of intracerebroventricular injections of the same medication, as well as with those of intravenous barbiturate and steroid anesthetics. Ketamine used intravenously initially decreased arterial blood pressure, but to a dose-dependent extent. This depression was short-lived and was occasionally followed by a milder phase. Intracerebroventricular injection of the drug provoked only a mild transient rise in mean arterial blood pressure. The intravenous injection of ketamine gave a brief period of respiratory depression, which was mirrored in the PaO2 and PaCO2 levels, followed by a period of respiratory stimulation with elevated PaO2 levels. The comparison of the three injection anesthetics revealed that barbiturate generated a much longer period of depression, whereas the blood gas tensions with ketamine indicated a brief period of respiratory depression similar to that seen with the steroid anesthetic. Blood gas tensions after the steroid anesthetic quickly reverted to normal, whereas those

after ketamine showed an elevated PaO2 following the first depression (Coulson *et al*., 1991)

2.4. Midazolam-Ketamine Combination

Benzodiazepines have minimal effects on the cardiovascular and respiratory systems and can be used for sedation in small ruminants and calves as the sole drug, but they are preferably used in combination with other agents. The use of benzodiazepines in cattle for standing sedation is not recommended, primarily because of the risk of inducing ataxia and recumbency; however, their muscle relaxant effects are beneficial when combined with ketamine for induction of anesthesia (Dyson *et al.*, 2023). Ketamine and midazolam or ketamine and diazepam combinations are commonly used since they have few adverse effects (Jacobson and Hartsfield, 1993) or no significant side effects on the cardiorespiratory system (Hellyer *et al*., 1991).

There were ten mature sheep, all of the same sex, weighing between 24 and 33 kg, and they seemed healthy. Intramuscular injections of ketamine (10 mg/kg BW), midazolam (1 mg/kg BW), and diazepam (1 mg/kg BW) were given in combination. Midazolam or diazepams, which are derivatives of benzodiazepines, were delivered first, and ketamine was injected ten minutes later. The experiment showed that the combination of midazolam and ketamine produced superior analgesia, muscular relaxation, and reliable surgical anesthesia in sheep when compared to diazepam and ketamine (Al-Redah, 2011).

Goats that received an intramuscular dose of 0.4 mg/kg of midazolam hydrochloride developed sedation and sternal recumbency. The administration of 1 mg/kg led to a fast onset of ataxia, lateral recumbency, and unconsciousness. The light surgical anesthetic was appropriate for non-painful procedures and lasted 7 to 15 minutes. While the respiratory rate increased only after midazolam at 0.4 mg/kg, the heart rate increased significantly ($p < 0.05$) at both dosage rates. Midazolam (0.4 mg/kg) and ketamine hydrochloride (4 mg/kg) together markedly elevated cardiac and respiratory rates ($p < 0.05$). For 16–39 minutes, a mild plane of surgical anesthesia appropriate for endotracheal intubation was induced (Stegmann, 1998).

2.5. Acepromazine-Ketamine Combination

There are several benefits to administering acepromazine along with ketamine. Acepromazine enhances the degree and duration of muscular relaxation, inhibits reflex limb movements, and lowers the dosage of ketamine required for a given length of analgesia. Acepromazine, however, extends standing and total recovery durations (Roberts *et al*., 2000).

Ketamine has a depressing effect on the myocardium, and it ends up stimulating the sympathetic nervous system (Ingwersen *et al.*, 1988). This action increases the heart rate, cardiac output and blood pressure (Pawson and Forsyth, 2010). These effects can be reduced with the use of an adjuvant drug, like acepromazine, because this drug is a tranquilizer that produces depression in the central nervous system, vasodilatation, and antiarrhythmic effects, and these effects have already been found in studies with dogs (Monteiro *et al.*, 2019).

After administering ketamine (11 mg/kg IV) for 15 minutes, six healthy sheep were given acepromazine (0.05 mg/kg IM), and throughout that time, their body temperatures consistently decreased. The heart rate decreased significantly at minutes 15, 30, 45, and 60 of the study. The mean arterial blood pressure significantly dropped between 30 and 45 minutes. The average respiratory rate fell sharply after 45 and 60 minutes. PaO2 significantly decreased after 5, 15, and 45 minutes, but PaCO2 increased at the 5-minute mark. There was a significant decrease in pH values at 5, 15, and 30 minutes (Baniadam *et al*., 2007).

Sheep were measured for pH, PaCO2, PaO2, standard bicarbonate, and base excess and lowered pH both before and after ketamine injections, both with and without atropine and acepromazine premedications. Fifteen minutes following the ketamine injection, measurements revealed a decrease in pH and PaO2, as well as a rise in PaCO2. PaCO2, PaO2, and pH did not significantly change when atropine was administered, either with or without acepromazine. There was no discernible difference in base excess, reduced pH, or normal bicarbonate values. This demonstrates how the blood buffer system of a

healthy animal counteracts any minor pH and PaCO2 changes that follow a ketamine infusion (Roberts *et al*., 2000).

CHAPTER III: MATERIALS AND METHOD

3.1. Study Area

The current study was carried out from July 2023 to October 2023 in Mekelle, Tigray, Ethiopia. Mekelle is the capital city of the Tigray region in northern Ethiopia. It is located approximately 780 kilometers north of the country's capital, Addis Ababa. Its coordinates are $13^0$32'N latitude and $39^0$33'E longitude, and its estimated population is 215,546. Its average elevation above sea level is 2200 meters, and its monthly minimum and maximum temperatures are 8.7 and 26.8 degrees Celsius, respectively. Mekelle receives 600 millimeters of rain a year on average, with July and August accounting for more than 70% of that total. The extended dry season lasts from October to May (CSA, 2011).

3.2. Study Population

In this study, a total of eight sheep were used for the experiment. The sheep in this study were mature and appeared to be in good health, with an average of 21.09±0.57 kg in weight and 2.69±0.15 years of age (mean±SD). It was declared healthy based on physiologically healthy measurements such as rectal temperature, heart rate and respiratory rate.

3.3. Sample Size

Eight adult, apparently healthy sheep (four males and four females) were used in the current study.

3.4. Study Design and Procedure

Experimental study design was conducted from July 2023 to October 2023 in the College of Veterinary Sciences at Mekelle University. Eight sheep were purchased, of which four were randomly assigned to an induction regimen of Midazolam-Ketamine (Group MK); the other four sheep were assigned to Acepromazine-Ketamine (Group AK).

3.4.1. Preparation of experimental animals

From the day before the animals were put under anesthesia, they were carefully watched. Before inducing balancing anesthesia, numerous clinico-physiological measurements were made. The animals that would be put under anesthesia the following morning were separated from the others and starved throughout the night. After washing with 10% povidone-iodine, blood was drawn through a jugular venipuncture prior to anesthesia. For convenient medication in following the anesthetic protocol, an intravenous catheter was fixed in the jugular vein.

3.4.2. Anesthetic drugs used

Group MK: In this study group, a combination of Midazolam-Ketamine at doses of 0.25 mg/kg and 10 mg/kg five minutes later was used, respectively.

Group AK: A combination of Acepromazine- Ketamine at doses of 0.5 mg/kg and 10 mg/kg five minutes later with disposable syringes (5 and 10 ml) was used for the intramuscular injection of the drugs.

3.4.3. Observation of post-intervention

After administration of Midazolam-Ketamine and Acepromazine-Ketamine, sheep from the two groups were kept under close observation. The heart rate was observed using a standard stethoscope. By examining how the abdominal muscles moved in the flank region, the respiratory rate was tracked. A digital thermometer was used to measure the rectal temperature. The heart rate and respiratory rate were computed using a watch. Additionally, a watch was utilized to keep track of how long each anesthetic parameter lasted from the time the anesthesia was induced until recovery was complete.

3.4.4. Evaluation of parameters

3.4.4.1. Monitoring of anesthesia

Onset of Anesthesia (in minutes)

This was done by recording the time (in minutes) of the initial injections of the drugs and the time of the disappearance of the pedal reflex. The difference between the former and the latter was the time of onset of anesthesia (Zhu *et al.*, 2016).

Duration of anesthesia (in minutes)

The duration of anesthesia was recorded as the time interval (in minutes) between the disappearance and return of the pedal reflex, as described by Udegbunam and Adetunji (2007).

Sternal recumbency time (in minutes)

Sternal recumbency time (SRT) was recorded as the time elapsed from the discontinuation of drug injections until the spontaneous regaining of sternal recumbency (Gautam *et al.*, 2018).

Standing Time (in minutes)

Standing time was calculated by determining the difference between the time of discontinuation of injection of drugs until the spontaneous regaining of standing position and the inability to walk (Gautam *et al.*, 2018).

Recovery (in minutes)

Recovery time was recorded as a time interval (in minutes) between the last bolus injection of Ketamine and the sheep's ability to stand without ataxia and walk (Zhu *et al.*, 2016).

3.4.4.2. Physiological parameters

The physiological parameters were recorded immediately before and after the administration of Midazolam-Ketamine and Acepromazine-Ketamine. The following physiological parameters were recorded:

Rectal temperature (°C)

The rectal temperature was measured in °C immediately before and after administration of Midazolam-Ketamine and Acepromazine-Ketamine. This was measured with a digital thermometer. The thermometer was inserted gently, at least 3cm, into the rectum in a rotatory manner, ensuring that the bulb was in contact with the mucus membrane of the rectum. Then, the thermometer was brought out to be read and recorded. This was done before the induction time of anesthesia and subsequently at every 10-minute interval following the induction until recovered.

Heart rate (beats/minute)

The heart rate (beats/minutes) was recorded immediately before and after the administration of Midazolam-Ketamine and Acepromazine-Ketamine. The heart rate was determined with the aid of a stethoscope. The sheep was placed on the table and made to lie down on its right side in a completely relaxed manner. A stethoscope was placed over the chest perpendicular to the apex of the heart, and the heartbeats were listened to through the earpiece. Each heartbeat was counted while looking at the already-set stopwatch to time the count. The heartbeats were counted for one-quarter of a minute, multiplied by four, and the result was recorded.

Respiratory rate (breaths/minute)

The respiratory rate (breaths/minutes) was recorded immediately before and after the administration of Midazolam-Ketamine and Acepromazine-Ketamine. This was done by visual observation and counting of thoraco-abdominal movement. Expiration and inspiration movements were counted as one cycle then respiratory rate was counted for half a minute multiplied by two. This was done before induction of the anesthesia and subsequently at every ten-minute interval post-induction.

3.4.4.3. Adverse effects

Adverse effects were observed by closely watching the sheep following the induction of the anesthesia. These were regurgitation, urination, defecation, salivation, apnea and ataxia.

3.4.4.4. Blood sample collection

Three ml of blood samples were collected from the veins of each sheep and immediately set in vacutainer tubes containing EDTA, and another 3 ml of blood was collected and set in the vacutainer tube free of anticoagulant to study the effect on hematological and biochemical parameters before and during anesthesia, respectively.

3.4.4.5. Hematological parameters

Three ml of blood samples were collected from the jugular vein of each experimental sheep before administration of the premedication and 30–40 minutes after administration of the midazolam-ketamine and Acepromazine-Ketamine because the maximum effects

of the Midazolam-Ketamine and Acepromazine-Ketamine occurred at 30–40 minutes. Immediately after collection, the blood samples were transferred into a sterile test tube with EDTA as an anticoagulant for estimation of packed cell volume, white blood cells, hemoglobin concentration, red blood cells, and differential leukocyte counts.

Hemoglobin (g/dL)

Hemoglobin was estimated by Sahli's hemoglobin meter as per the standard method recommended by Orpet and Welsh (2001). The values were expressed in *g/dL*.

Packed Cell Volume (%)

Packed cell volume was estimated by microhematocrit as described by Feldman *et al* (2000), and the values were expressed in percentage.

Total Erythrocyte Count ($\times 10^6/\mu L$)

The total erythrocyte count was estimated as per the procedure described by Orpet and Welsh (2001) using Neubauer's slide, and the values were expressed in million cells per microliter of blood.

Total Leukocyte Count ($\times 10^3/\mu L$)

The total leukocyte count was estimated as per the procedure described by Jain *et al* (2000), and the values were expressed as thousand cells per microliter of blood.

Differential Leukocyte Count (%)

The differential leukocyte count was estimated by staining the blood smear with Giemsa stain, and 100 leukocytes were counted using the battlement method described by Jain *et al* (2000). The differential leukocyte count was expressed in percentages. These were neutrophils, lymphocytes, monocytes, eosinophils and basophils.

3.4.4.6. Biochemical parameters

Blood samples were collected in plain tubes via jugular vein puncturing with a 23-G needle from each experimental sheep prior to administration of the premedication and thirty minutes after administration of the midazolam-ketamine and acepromazine-ketamine during the observation period. After collection, the blood was centrifuged to harvest the serum and stored at -20 °C. The biochemical parameters were estimated by an auto-analyzer, and the values were expressed in mg/dL from the separated serum before

and during anesthesia. These were serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), creatinine, and serum glucose level.

3.5. Data Collection

The physiological parameters (heart rate, respiratory rate, and rectal temperature), the anesthetic indices (induction period, duration of anesthesia, sternal recumbency, standing time, and recovery period), the hematological parameters (packed cell volume, red blood cells, white blood cells, hemoglobin concentration, and differential leukocyte counts), and the biochemical parameters (AST, ALT, creatinine, and plasma glucose level) were all recorded prior to and after anesthetic combination administration.

3.6. Data Analysis

The data was entered into a Microsoft Excel spreadsheet and analyzed using SPSS version 23.0 (Winer, 2001), which calculates the mean and SD (standard deviation) for each variable. Comparing physiological and hematological measurements made prior to and throughout the administration of the drug combination for each group was done using a paired t-test. To pinpoint the negative impacts, Fisher's exact test was applied. The level of significance of the mean values of the anesthetic indices between two groups was assessed using an independent t-test at a 95% confidence level. A statistically significant value was one with a p-value less than 0.05.

3.7. Ethical Consideration

The College of Veterinary Sciences' Animal Ethics and Experimentation Committee (AEEC) granted ethical approval, and the experiment took into account the Guidelines on Care and Use of Animals for Scientific Purposes**.**

CHAPTER IV: RESULTS

4.1. Anesthetic Indices of the Combinations

The mean (±SD) induction times in minutes were 15.10 ± 4.28 and 7.85 ± 3.73 in MK and AK, respectively. There was a statistically significant ($p = 0.043$) difference in induction time between the two groups. Thus, induction time in group MK was longer than in AK (Table 1).

The mean (±SD) time for the duration of anesthesia in minutes was 43.03 ± 1.12 and 17.01 ± 6.27 in MK and AK, respectively. The duration of anesthesia was longer in MK than in AK and was statistically significant ($p = 0.003$) (Table 1).

The mean (± SD) time for sternal recumbency in minutes was 5.53 ± 0.22 and 4.17 ± 0.06 in MK and AK, respectively. The time for sternal recumbency in MK was longer than in AK and statistically significant ($p = 0.001$) (Table 1).

The mean (±SD) time for unassisted standing in minutes was 44.93 ± 1.40 and 22.34 ± 5.91 in MK and AK, respectively. The time for standing was significantly ($p = 0.003$) longer in MK than in AK (Table 1).

The mean (±SD) time for the duration of recovery in minutes was 49.89 ± 5.10 and 33.61 ± 5.92 in MK and AK, respectively. The recovery duration was longer in MK than in AK and was statistically significant ($p = 0.006$) (Table 1).

Table 1: Anesthetic effects of the two combinations

Anesthetic combination	Dose	Onset of action	Duration of anesthesia	Sternal recumbency time	Standing time	Recovery period
MK(G1)	0.25 mg/kg and 10 mg/kg	15.10± 4.28	43.03±1.12	5.53±0.22	44.93 ±1.40	49.89±5.10
AK(G2)	0.5 mg/kg and 10 mg/kg	7.85±3. 73	17.01±6.27	4.17±0.64	22.34 ±5.91	33.61±5.92

4.2. Effects of Midazolam and Ketamine Combination on Physiological Parameters

There was no significant difference in rectal temperature in MK and AK ($p = 0.123$) before anesthesia. The rectal temperature significantly decreased until 30 minutes ($p = 0.001$) and insignificantly increased ($p = 0.408$) thereafter. The heart rate ($p = 0.010$) and respiratory rate ($p = 0.018$) were also significantly increased after administration of the anesthetic combination in the MK group (Table 2).

Table 2: Effects of Midazolam and Ketamine combination on rectal temperature, heart rate and respiratory rate

	MK		
Time in minutes	RT(0c)	HR(b/min)	RR(c/min)
BA	38.53±0.39	69.00±2.58	23.00±2.58
10	38.1±0.45	71.00±2.58	25.00±2.58
20	37.85±0.39	73.50±3.00	27.50±2.52
30	37.58±0.42	76.00±2.31	28.50±1.92
40	37.43±0.33	78.50±3.42	30.00±1.63
50	37.68±0.28	80.00±2.83	31.50±1.00
60	37.78±0.30	81.50±2.52	33.00±1.16

BA= Before anesthesia, RT= Rectal temperature, HR= Heart rate, RR= Respiratory rate

4.3. Effects of the Acepromazine- Ketamine Combination on Physiological Parameters

The recorded rectal temperature significantly decreased until 30 minutes ($p = 0.038$) and insignificantly increased ($p = 0.245$) thereafter. The heart rate decreased significantly until 30 minutes ($p = 0.014$), then increased significantly ($p = 0.014$). The respiratory rate significantly increased until thirty minutes ($p = 0.608$), then insignificantly decreased ($p = 1.000$) following the administration of the anesthetic combination in the AK group (Table 3).

Table 3: Effects of the Acepromazine and Ketamine combination on rectal temperature, heart rate and respiratory rate

	AK		
Time in minutes	RT(0c)	HR(b/min)	RR(c/min)
BA	39.03±0.40	73.00±2.58	29.50±2.52
10	38.73±0.43	68.00±4.62	30.50±1.92
20	38.38±0.21	59.00±6.83	30.50±4.12
30	37.98±0.22	62.00±6.93	29.00±4.76
40	38.63±0.75	63.50±9.15	26.00±3.65
50	38.25±0.64	65.00±7.02	27.00±2.58
60	38.43±0.77	65.00±7.02	29.00±2.58

BA= Before anesthesia; RT= Rectal temperature; HR= Heart rate; RR= Respiratory rate

4.4. Adverse Effects of Ketamine Combinations

Adverse effects observed included urination, defecation, salivation, ataxia and apnea. There was no regurgitation in both groups. Four sheep (two from each group) salivated in both groups. Ataxia was observed in two of the four sheep in MK and one sheep in AK. Two sheep in both MK and AK defecated during anesthesia, while three sheep in MK and two sheep in AK urinated during anesthesia. One sheep in each group showed apnea (Table 4).

Table 4: Adverse effects of the combinations

Adverse effects	**MK(4)**	**AK(4)**
Salivation	2 (50%	2 (50%)
Urination	2(50%)	3 (50%)
Defecation	2 (50%)	2 (50%)
Ataxia	1 (25%)	2 (50%)
Apnea	1(25%)	1 (25%)

4.5. Effects of the two Ketamine-Anesthetic Combinations on Hematological Parameters

In the MK group, hemoglobin concentration (p = 0.3416) had significantly increased. However, packed cell volume (p = 0.0038), total erythrocyte count (p = 0.0014), lymphocytes (p = 0.0022), monocytes (p = 0.0058), eosinophils (p = 0.0034), and basophils (p = 0.0060) had significantly decreased. But the total leucocyte count (p = 0.0167) and neutrophil count (p = 0.0058) had significantly increased (Table 5).

In the AK group, packed cell volume (p = 0.0285), total erythrocyte count, and neutrophils (p = 0.0176) had significantly increased. Whereas hemoglobin concentration (p = 0.0099), total leucocyte count (p = 0.0049), lymphocytes (p = 0.0064), monocytes (p = 0.0003), eosinophils (p = 0.0023), and basophils (p = 0.0319) had significantly decreased (Table 5).

Table 5: Effects of the two combinations on hematological parameters

AC	**TI**	**Hematological parameters**								
		HBC	PCV	TEC	TLC	NTP	LYM	MN	EOS	BAS
MK	BA	9±0.37	29.25±0.96	11.75±0.34	7.48±0.28	31.7±1.6	54.68±1.94	3.35±0.21	7.5±0.26	0.75±0.13
	DA	9.25±0.62	21.5±1.29	11±0.39	8.93±0.84	33.65±1.56	52.23±1.74	2.85±0.17	6.9±0.32	0.58±0.42
AK	BA	10.3±0.18	29.48±1.09	8.63±0.13	10.95±0.58	28.73±0.53	56.7±o.99	3.28±0.15	7.3±0.26	0.78±0.10
	DA	9.43±0.17	31.05±0.91	9.5±0.26	10.3±0.66	33.23±1.4	53.85±0.64	2.33±0.22	6.5±0.26	0.45±0.06

AC= Anesthetic combination; TI=Time interval; BA= Before anesthesia; DA= During anesthesia; HBC = Hemoglobin concentration; PCV = Packed cell volume; TEC = Total erythrocyte count; TLC = Total leukocyte count; NTP = Neutrophil; LYM = Lymphocyte; MON = Monocyte; EOS = Eosinophil; BAS = Basophil

3.6. Effects of the two Ketamine Combinations on Biochemical Parameters

In the MK group, aspartate aminotransferase (p = 0.0006), alanine aminotransferase (p = 0.0121), creatinine (p = 0.0029), and serum glucose level (p = 0.0027) had significantly increased (Table 6).

In the AK group, aspartate aminotransferase (p=0.5295) decreased insignificantly. Alanine aminotransferase (p=0.1644) and serum glucose level (p=0.2788) had insignificantly increased. Creatinine had significantly decreased (p=0.0121) (Table 6).

Table 6: Effects of the two combinations on biochemical parameters

AC	**TI**	**Biochemical parameters**			
		AST	ALT	CRE	SGL
MK	BA	93.50±7.49	11.88±0.49	1.50±0.06	40.8±6.80
	DA	95.43±7.61	17.33±2.15	1.52±0.05	45.33±7.71
AK	BA	81.28±17.52	15.23±5.50	0.59±0.15	24.85±5.00
	DA	78.33±13.13	18.55±8.62	0.52±0.16	30.35±7.45

AC=Anesthetic combination; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; CRE= Creatinine; SGL= Serum glucose level

CHAPTER V: DISCUSSION

The effects of ideal balanced anesthesia are mild or nonexistent and include sleep, amnesia, analgesia, and muscle relaxation. Before administration of the anesthetic compounds, sedative and analgesic drugs should be given to induce anesthesia in sheep (Malik, 2014). Ketamine is rarely used alone because it does not effectively relax muscles or cause tachycardia, catalepsy, or muscle rigidity. Instead, it is often used in conjunction with midazolam and acepromazine to minimize side effects. Ketamine is a dissociative anesthetic that is typically used in conjunction with alpha-2 agonists or benzodiazepines to reduce the negative effects on the animal and reduce stress (Marjani *et al*., 2015). Opioids, associated with tranquilizers or sedatives, are also part of these preanesthetic protocols, providing analgesia and adequate sedation for different types of surgeries and veterinary procedures (Killos *et al.*, 2010).

In this study, the induction, duration of anesthesia, sternal recumbency, standing, and recovery time in the MK group were significantly longer than those in the AK group. This was in agreement with the experiment done by Udegbunam and Adetunji (2007), in which induction of anesthesia was shortest during the administration of Acepromazine-Ketamine in goats, and with Asif *et al* (2021), who found the combined therapy with detomidine midazolam-propofol-ketamine resulted in an insignificant increase in the duration of anesthesia in goats.

In the present study, rectal temperature was significantly decreased until 30 minutes and insignificantly increased thereafter. The heart rate and respiratory rate had also significantly increased after administration of the anesthetic combination in the MK group. This is in contravention of the experiment done by Al-Redah (2011), in which the respiratory rate was gradually reduced during the first 20 minutes of Midazolam-Ketamine administration to reach 27-33 breaths per minute at 30-45 minutes, then gradually increased to reach 41-44 breaths per minute at 60-70 minutes. The heart rate peaked at 132 beats per minute at 5 minutes after IM injection, then gradually decreased to 120 beats per minute at 20 minutes, then increased to nearly 120-122 beats per minute from 30 minutes to 75 minutes, and in agreement with the decline in rectal temperature. The decrease in rectal temperature probably occurred as a result of the administration of

benzodiazepines derivative (midazolam) because of central nervous system depression and a reduction in muscular activity (Kiliç, 2008). The recent study was in relation to research done by Stegmann (1998), following sedation with midazolam (0.4 mg/kg), the administration of ketamine increased the heart rate significantly ($p < 0.05$) from 75 (±11) to 106 (±13) beats per minute in goats.

In this study, the recorded rectal temperature significantly decreased until 30 minutes and insignificantly increased thereafter. The heart rate decreased significantly until 30 minutes, then increased significantly. The respiratory rate had insignificantly increased until 30 minutes, and then decreased following the administration of the anesthetic combination in the AK group. This was in agreement with the study by Baniadam *et al* (2007), in which rectal temperature declined at 5 to 60 minute and mean heart rate declined from 84.17 beats per minute to 63.83 beats per minute, and then increased to 74.5 beats per minute at 30 and 60 minute intervals, respectively after the Acepromazine-Ketamine injection. At the 15-60 minute intervals, a significant decrease in heart rate was observed in sheep given atropine-acepromazine-ketamine anesthesia as compared to animals given ketamine alone or in conjunction with atropine, as acepromazine inhibits the pressor response to epinephrine (Stegmann, 1998). At five minutes the mean respiratory rate increased and then declined gradually at 15-60 minutes post injection intervals. The respiratory rate values at 45 and 60 minutes were significantly lower than before the administration. The mean rectal temperature declined significantly from baseline at all-time points. The mean temperature increased at 60 minutes but did not reach the baseline following the administration of acepromazine and ketamine combination (Grimm *et al.*, 2017).

In the present study, there was apnea, salivation, and urination following injections of the acepromazine-ketamine combination. This breathing pattern has been reported following the sole use of ketamine in sheep (Roberts *et al*., 2000). These adverse effects have also been documented in previous studies that have used ketamine medication combinations (Carroll and Hartsfield, 1996). Similarly, a recent experiment done by Asif *et al* (2021) showed urination and mild ataxia in animals treated with midazolam-ketamine.

In this study, there was a significant decrease in hemoglobin concentration in the AK group. This was consistent with the study by Sciences (2016), in which a significant decrease in HBC was observed at 15 minutes of acepromazine administration which further decreased at five minutes of administration of ketamine and remained significantly lower at recovery as compared to the base value. There was a significant decrease in PCV at 15 min of acepromazine administration, at five minutes of ketamine administration and at recovery as compared to the base value in buffalo calves. This was in contrast to the present study, where there was a significant increase in packed cell volume in the AK group. During anesthesia or sedation, the decrease in PCV and HBC is attributed to the shifting of fluid from the extravascular compartment to the intravascular compartment to maintain normal cardiac output in animals. This decrease in HBC could also be attributed to the splenic pooling of blood constituents (Hewson *et al*., 2006).

In this study, serum glucose levels had insignificantly increased in the AK group, which was the same as the study by Sciences (2016), who reported that after administering acepromazine for 15 minutes, there was a considerable increase in the serum glucose level. As ketamine was administered for five minutes, there was a noticeable hyperglycemia, which rose even more during recovery as compared to the baseline in buffalo calves. The release of catecholamine under stressful conditions may be the cause of the glycogenolysis that results in hyperglycemia during anesthesia (Kilic, 2008).

Throughout the observation period, the majority of the time intervals for serum glucose level readings in the groups showed an upward trend. An alpha-2 adrenergic suppression of insulin produced by pancreatic beta cells as well as enhanced glucose synthesis in the liver may be responsible for the rise in serum glucose seen in the current study. Another possible cause of hyperglycemia is the release of more adrenocorticotropic hormones as a result of acute stress, increased muscular activity, and sympathetic stimulation brought on by the restraint of the animals. In this study; there was a significant increase in AST, ALT and serum glucose levels following administration of both groups. There might be a synergistic effect on liver enzymes and glucose metabolism. The combined administration of these drugs can potentially result in a greater impact on liver function compared to using them individually, leading to increased AST and ALT levels. The

administration of anesthesia, including midazolam, acepromazine, and ketamine, can induce a stress response in animals. This stress response can lead to the release of stress hormones such as cortisol, which can affect glucose metabolism in the body. Increased serum glucose levels may be observed as a result of increased gluconeogenesis (glucose production) in the liver (Sobti *et al.*, 1981).

6. CONCLUSION AND RECOMMENDATIONS

The results of this study revealed that the combination of Acepromazine-Ketamine produced a faster onset of action than the combination of Midazolam-Ketamine, whereas the duration of action and recovery time were longer in Midazolam-Ketamine than Acepromazine-Ketamine combination. Additionally, the findings indicated that in both the groups physiological and hematobiochemical parameters underwent a considerable change during anesthesia. Acepromazine-Ketamine was determined to be a better general anesthetic combination for quick onset and shorter duration of anesthesia whereas Midazolam- Ketamine was a suitable choice for longer duration of anesthesia.

Therefore, the following recommendations are forwarded based on the present findings:

- Ketamine should never be taken alone during surgery; always utilize sedatives or tranquilizers first.
- Midazolam-Ketamine and Acepromazine-Ketamine anesthetic combinations should be practiced in sheep for different surgical procedures.
- Additional study is required to properly evaluate and document the anesthetic effect of these combinations in the clinico-physiological and hematobiochemical parameters.

7. REFERENCES

Adams, H.A. (1997) '[S-(+)-ketamine. Circulatory interactions during total intravenous anesthesia and analgesia-sedation]', *Der Anaesthesist*, 46(12), pp. 1081–7.

Al-Redah, S.A.A. (2011) 'A comparative study between using of Midazolam-Ketamine and Diazepam-Ketamine combinations as anaesthetic program in sheep', *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 10(1), p. 66. Available at: https://doi.org/10.29079/vol10iss1art145.

Ashraf, M.B., Antora Akter, M., Saha, M., Mishra, P., Hoda, N. and Alam, M.M. (2019) 'Clinicopathological Evaluation On Capture Myopathy Due To Chemical Immobilization In Spotted Deer', *Turkish Journal of Veterinary Research*, 3(2), pp. 73–79. Available at: http://www.dergipark.gov.tr/tjvr.

Asif, M., Khan, M.A., Akbar, H. and Omer, M.O. (2021) 'Effects of detomidine and midazolam administered alone or combined, on induction dose and quality of propofol and ketamine anesthesia in goats', *Journal of Animal and Plant Sciences*, 31(2), pp. 441–449. Available at: https://doi.org/10.36899/JAPS.2021.2.0233.

Baniadam, A., Afshar, F.S. and Balani, M.R.B. (2007) 'Cardiopulmonary effects of acepromazine-ketamine administration in the sheep', *Bulletin of the Veterinary Institute in Pulawy*, 51(1), pp. 93–96.

Bustamante, R. and Valverde, A. (1997) 'Determination of a sedative dose and influence of droperidol and midazolam on cardiovascular function in pigs', *Canadian Journal of Veterinary Research*, 61(4), pp. 246–250.

Carroll, G.L. and Hartsfield, S.M. (1996) 'General anesthetic techniques in ruminants.', *The Veterinary clinics of North America. Food animal practice*, 12(3), pp. 627–661. Available at: https://doi.org/10.1016/S0749-0720(15)30391-1.

Chambers, J.P. and Dobson, J.M. (1989) 'A midazolarn and ketamine combination as a sedative in cats', *Veterinary Anaesthesia and Analgesia*, 16(1), pp. 53–54. Available at: https://doi.org/10.1111/j.1467-2995.1989.tb00073.x.

Clarke, K.W. (Kathy W.., Hall, L.W. and Trim, C.M. (Cynthia M.. (2013) 'Veterinary Anaesthesia E-Book', p. 612.
Available athttps://books.google.com/books?id=hZh5AAAAQBAJ&pg=PA612.

Clifford, D.H. (1984) 'Preanesthesia, Anesthesia, Analgesia, and Euthanasia', *Laboratory Animal Medicine*, pp. 527–562.
Available at: https://doi.org/10.1016/b978-0-12-263620-2.50024-0.

Coulson, N.M., Januszkiewicz, A.J. and Ripple, G.R. (1991) 'Physiological responses of sheep to two hours anaesthesia with diazepam-ketamine', *Veterinary Record*, 129(15), pp. 329–332.
Available at: https://doi.org/10.1136/vr.129.15.329.

CSA (2011) 'Report om Annual Average Retail Prices of Goods and Services'.
Available at: www.csa.gov.et.

Dunlop, C.I. and Hoyt, R.F. (1997) 'Anesthesia and Analgesia in Ruminants', *Anesthesia and Analgesia in Laboratory Animals*, pp. 281–311.
Available at: https://doi.org/10.1016/b978-012417570-9/50016-7.

Dyson, M.C., Jirkof, P., Lofgren, J., Nunamaker, E.A. and Pang, D. (2023) 'Anesthesia and Analgesia in Laboratory Animals', *Anesthesia and Analgesia in Laboratory Animals*, pp. 1–762.
Available at: https://doi.org/10.1016/C2019-0-04422-6.

Feldman, B.F., Zinkl, J.K. and Jain, N.C. (2000) 'Schalm's Veterinary Haematology', pp. 1344–1348.
Availableat:https://www.scirp.org/%28S%28351jmbntvnsjt1aadkposzje%29%29/reference/referencespapers.aspx?referenceid=2645457.

Gautam, D., Kinjavdekar, P., Amarpal, Aithal, H.P., Pawde, A.M., Kumar, R., Sivanarayan, T.B., Mohan, D. and Singh, R. (2018) 'Evaluation of dexmedetomidine alone and its combination with ketamine and ropivacaine for tube cystostomy in urolithic buffalo calves', *Buffalo Bulletin*, 37(2), pp. 199–209.

Goodchild, C. and Noble, J. (1987) 'The effects of intrathecal midazolam on sympathetic nervous system reflexes in man-a pilot study.', *British Journal of Clinical Pharmacology*, 23(3), pp. 279–285.

Available at: https://doi.org/10.1111/j.1365-2125.1987.tb03046.x.

Goodchild, C.S. and Serrao, J.M. (1987) 'Intrathecal midazolam in the rat: Evidence for spinally-mediated analgesia', *British Journal of Anaesthesia*, 59(12), pp. 1563–1570.

Available at: https://doi.org/10.1093/bja/59.12.1563.

Grimm, K.A., Lamont, L.A., Tranquilli, W.J., Greene, S.A. and Robertson, S.A. (2017) 'Veterinary Anesthesia and Analgesia: The Fifth Edition of Lumb and Jones', *Veterinary Anesthesia and Analgesia: The Fifth Edition of Lumb and Jones*, pp. 1–1061.

Available at: https://doi.org/10.1002/9781119421375.

Hayashi, K., Nishimura, R., Yamaki, A., Hwi-Kim, Y., Matsunaga, S., Sasaki, N. and Takeuchi, A. (1994) 'Comparison of Sedative Effects Induced by Medetomidine, Medetomidine-Midazolam and Medetomidine-Butorphanol in Dogs', *Journal of Veterinary Medical Science*, 56(5), pp. 951–956.

Available at: https://doi.org/10.1292/jvms.56.951.

Hellyer, P.W., Freeman, L.C. and Hubbell, J.A.E. (1991) 'Induction of Anesthesia with Diazepam-Ketamine and Midazolam-Ketamine in Greyhounds', *Veterinary Surgery*, 20(2), pp. 143–147.

Available at: https://doi.org/10.1111/j.1532-950X.1991.tb00324.x.

Hewson, C.J., Dohoo, I.R. and Lemke, K.A. (2006) 'Perioperative use of analgesics in dogs and cats by Canadian veterinarians in 2001', *Canadian Veterinary Journal*, 47(4), pp. 352–359.

Ingwersen, W., Allen, D.G., Dyson, D.H., Pascoe, P.J. and O'Grady, M.R. (1988) 'Cardiopulmonary effects of a ketamine hydrochloride/acepromazine combination in healthy cats', *Canadian Journal of Veterinary Research*, 52(1), pp. 1–4.

Jacobson, J.D. and Hartsfield, S.M. (1993) 'Cardiovascular effects of intravenous bolus administration and infusion of ketamine-midazolam in isoflurane-anesthetized dogs', *American Journal of Veterinary Research*, 54(10), pp. 1715–1720.

Jain, A.K., Duin, R.P.W. and Mao, J. (2000) 'Statistical pattern recognition: A review', *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 22(1), pp.

4–37.
Available at: https://doi.org/10.1109/34.824819.

Johansen, M.J., Gradert, T.L., Satterfield, W.C., Baze, W.B., Hildebrand, K., Trissel, L. and Hassenbusch, S.J. (2004) 'Safety of continuous intrathecal midazolam infusion in the sheep model', *Anesthesia and Analgesia*, 98(6), pp. 1528–1535. Available at: https://doi.org/10.1213/01.ANE.0000120086.35289.9D.

Kiliç, N. (2008) 'Cardiopulmonary, biochemical, and haematological changes after detomidine-midazolam-ketamine anaesthesia in calves', *Bulletin of the Veterinary Institute in Pulawy*, 52(3), pp. 453–456.

Killos, M.B., Graham, L.F. and Lee, J. (2010) 'Comparison of two anesthetic protocols for feline blood donation', *Veterinary Anaesthesia and Analgesia*, 37(3), pp. 230–239.
Available at: https://doi.org/10.1111/j.1467-2995.2010.00527.x.

Leece, E. (2017) *Veterinary Anesthesia and Analgesia*, *Veterinary Anaesthesia and Analgesia*. Available at: https://doi.org/10.1016/j.vaa.2016.06.009.

Mahmud, M.A., Shaba, P., Yisa, H.Y., Gana, J., Ndagimba, R. and Ndagi, S. (2014) 'Comparative efficacy of Diazepam, Ketamine, and Diazepam-Ketamine combination for sedation or anesthesia in cockerel chickens', *Journal of Advanced Veterinary and Animal Research*, 1(3), pp. 107–113.
Available at: https://doi.org/10.5455/javar.2014.a24.

Malik, V. (2014) 'An update on general anaesthesia in ruminants', *Indian Journal of Veterinary Surgery*, 35(1), pp. 1–11.

Marjani, M., Akbarinejad, V. and Bagheri, M. (2015) 'Comparison of intranasal and intramuscular ketamine-midazolam combination in cats', *Veterinary Anaesthesia and Analgesia*, 42(2), pp. 178–181.
Available at: https://doi.org/10.1111/vaa.12183.

Marland, S., Ellerton, J., Andolfatto, G., Strapazzon, G., Thomassen, O., Brandner, B., Weatherall, A. and Paal, P. (2013) 'Ketamine: Use in Anesthesia', *CNS Neuroscience and Therapeutics*, 19(6), pp. 381–389.
Available at: https://doi.org/10.1111/cns.12072.

Michaloudis, D.G., Kanakoudis, F.S., Xatzikraniotis, A. and Bischiniotis, T.S. (1995)

‘The effects of midazolam followed by administration of either vecuronium or atracurium on the QT interval in humans’, *European Journal of Anaesthesiology*, 12(6), pp. 577–583.

Mohiuddin, M., Hasan, M., Shohag, M., Ferdousy, R.N., Alam, M.M. and Juyena, N.S. (2018) ‘Surgical management of limb fractures in calves and goats’, *Bangladesh Veterinary Journal*, 52(1–4), pp. 46–56.
Available at: https://doi.org/10.32856/bvj-2018.06.

Monteiro, E.R., Rabello, T.A., Rangel, J.P.P., Nunes, J.S., Freire, C.D. and Campagnol, D. (2019) ‘Effects of 3 morphine doses, in combination with acepromazine, on sedation and some physiological parameters in dogs’, *Canadian Journal of Veterinary Research*, 83(3), pp. 235–240.

Nishimura, R., Kim, H.Y., Matsunaga, S., Hayashi, K., Sasaki, N., Takeuchi, A. and Tamura, H. (1994) ‘Cardiopulmonary Effects of Medetomidine-Midazolam and Medetomidine-Midazolam-Atipamezole in Laboratory Pigs’, *Journal of Veterinary Medical Science*, 56(2), pp. 375–377.
Available at: https://doi.org/10.1292/jvms.56.375.

Orpet, H. and Welsh, P. (2001) ‘Handbook of Veterinary Nursing’, *Handbook of Veterinary Nursing* [Preprint].
Available at: https://doi.org/10.1002/9780470690376.

Pawson, P. and Forsyth, S. (2010) ‘Agentes anestésicos’, *Farmacologia clínica de pequenos animais*, pp. 83–112.

Reves, J.G., Corssen, G. and Holcomb, C. (1978) ‘Comparison of two benzodiazepines for anaesthesia induction: midazolam and diazepam’, *Canadian Anaesthetists’ Society Journal*, 25(3), pp. 211–214.
Available at: https://doi.org/10.1007/BF03004881.

Roberts, B.R., Livingston, A. and Waterman-Pearson, A.E. (2000) ‘Antinociceptive effects of intrathecal 5-HT agonists in sheep’, *Veterinary Anaesthesia and Analgesia*, 27(2), pp. 73–81.
Available at: https://doi.org/10.1046/j.1467-2995.2000.00020.x.

Rubio, M., Satué, K., Carrillo, J.M., Guerra, Á.H., Cuervo, B., Chicharro, D., Damiá, E., Del Romero, A. and Sopena, J. (2022) ‘Changes in Hematological and

Biochemical Profiles in Ovariohysterectomized Bitches Using an Alfaxalone–Midazolam–Morphine–Sevoflurane Protocol', *Animals*, 12(7). Available at: https://doi.org/10.3390/ani12070914.

Salonen, M., Onaivi, E.S. and Maze, M. (1992) 'Dexmedetomidine synergism with midazolam in the elevated plus-maze test in rats', *Psychopharmacology*, 108(1–2), pp. 229–234. Available at: https://doi.org/10.1007/BF02245313.

Sciences, A. (2016) 'Research Article evaluation of acepromazine-ketamine anaesthesia in buffalo calves', 55(1), pp. 76–79.

Serrao, J.M., Marks, R.L., Morley, S.J. and Goodchild, C.S. (1992) 'Intrathecal midazolam for the treatment of chronic mechanical low back pain: a controlled comparison with epidural steroid in a pilot study', *Pain*, 48(1), pp. 5–12. Available at: https://doi.org/10.1016/0304-3959(92)90125-U.

Simon, B.T., Scallan, E.M., Siracusa, C., Henderson, A., Sleeper, M.M. and Menzies, M.P.L. (2014) 'Effects of acepromazine or methadone on midazolam-induced behavioral reactions in dogs', *Canadian Veterinary Journal*, 55(9), pp. 875–885.

Skarda, R.T. (1996) 'Local and regional anesthesia in ruminants and swine.', *The Veterinary clinics of North America. Food animal practice*, 12(3), pp. 579–626. Available at: https://doi.org/10.1016/S0749-0720(15)30390-X.

Smith, A.C., Zellner, J.L., Spinale, F.G. and Swindle, M.M. (1991) 'Sedative and cardiovascular effects of midazolam in swine', *Laboratory Animal Science*, 41(2), pp. 157–161.

Sobti, V.K., Mirakhur, K.K., Krishnamurthy, D. and Nigam, J.M. (1981) 'Ringer's lactate & homologous blood transfusion in experimental haemorrhagic shock in buffalo calves (Bubalus bubalis)', *Indian Journal of Experimental Biology*, 19(4), pp. 371–374.

Stegmann, G.F. (1998) 'Observations on the use of midazolam for sedation, and induction of anaesthesia with midazolam in combination with ketamine in the goat', *Journal of the South African Veterinary Association*, 69(3), pp. 89–92.

Available at: https://doi.org/10.4102/jsava.v69i3.823.

Tammisto, T., Jäättelä, A., Nikki, P. and Takki, S. (1971) 'Effect of pentazocine and pethidine on plasma catecholamine levels.', *Annals of Clinical Research*, 3(1), pp. 22–29.

Toholj, B.D., Kuja, V.D., Stevan, M.R., Spasojevi, J.M. and Smolec, O.B. (2014) 'Case Report the use of ketamine , xylazine and midazolam combination for total intravenous anesthesia (tiva) in surgical removal of abdominal testis at stallion', 37(2), pp. 185–188.

Tranquilli, W.J., Graning, L.M., Thurmon, J.C., Benson, G.J., Moum, S.G. and Lentz, E.L. (1991) 'Effect of midazolam preanesthetic administration on thiamylal induction requirement in dogs', *American Journal of Veterinary Research*, 52(5), pp. 662–664.

Tranquilli, W.J., Thurmon, J.C. and Grimm, K.A. (2007) *Veterinary Anesthesia and Analgesia 'Lumb & Jones'*.

Udegbunam, R.I. and Adetunji, A. (2007) 'Comparison of three Ketamine drug combinations for short term anaesthesia in West African Dwarf Goats', *Agro-Science*, 6(2).

Available at: https://doi.org/10.4314/as.v6i2.1573.

Vineet, B. and Bharat, S. (2007) 'Midazolam as tranquilizer in dogs', *Indian Veterinary Journal*, 84(11), pp. 1141–1145.

Winer, R.S. (2001) 'A framework for customer relationship management', *California Management Review*, (4), pp. 89–105.

Available at: https://doi.org/10.2307/41166102.

Zhu, J., Meza, J., Kato, A., Saedi, A., Chetan, D., Parker, R., Caldarone, C.A., McCrindle, B.W., Van Arsdell, G.S. and Honjo, O. (2016) 'Pulmonary flow study predicts survival in pulmonary atresia with ventricular septal defect and major aortopulmonary collateral arteries', *Journal of Thoracic and Cardiovascular Surgery*, 152(6), pp. 1494-1503.e1.

Available at: https://doi.org/10.1016/j.jtcvs.2016.07.082.

8. ANNEXES

I. Map of study area

Source: Figure - available from: BJOG An International Journal of Obstetrics & Gynaecology

Fig 1: Map of study area

II. Ethical clearance

Ous - 0998/0.1
03/11/2013.

Mekelle University

Animal Ethics and Experimentation Committee (AEEC) Clearance Letter

To: Hailay Kahsay, Principal Investigator

Mekelle

Date: 09/10//2015 E.C

Subject: Notification of AEEC decision on your research proposal

AEEC No: AEEC 03/2015 E.C

Protocol: : *Effect of midazolam and acepromazine with ketamine combination on physiological and hematobiochemical parameters on sheep*

Dear PI

The aforementioned research proposal which was submitted by you for animal ethics and use clearance letter has been reviewed by the Animal Ethics and Experimentation Committee (AEEC).

The AEEC has discussed and examined the research proposal in detail from animal ethics and use principles and values perspective.

Finally, the AEEC has (**approved**, approved with modification or withhold approval) your research proposal.

This animal ethics clearance letter is valid for only three months (14/11/15 E.C – 15/12/15 E.C.).

Furthermore, any other correspondence and inquiries concerning you research proposal with committee must include the AEEC No., the name of the PI and the proposal title.

Best Regards

AEEC Chairperson

Name: Dr Enquebaher Kassaye (PhD)

Signature:

AEEC Secretary

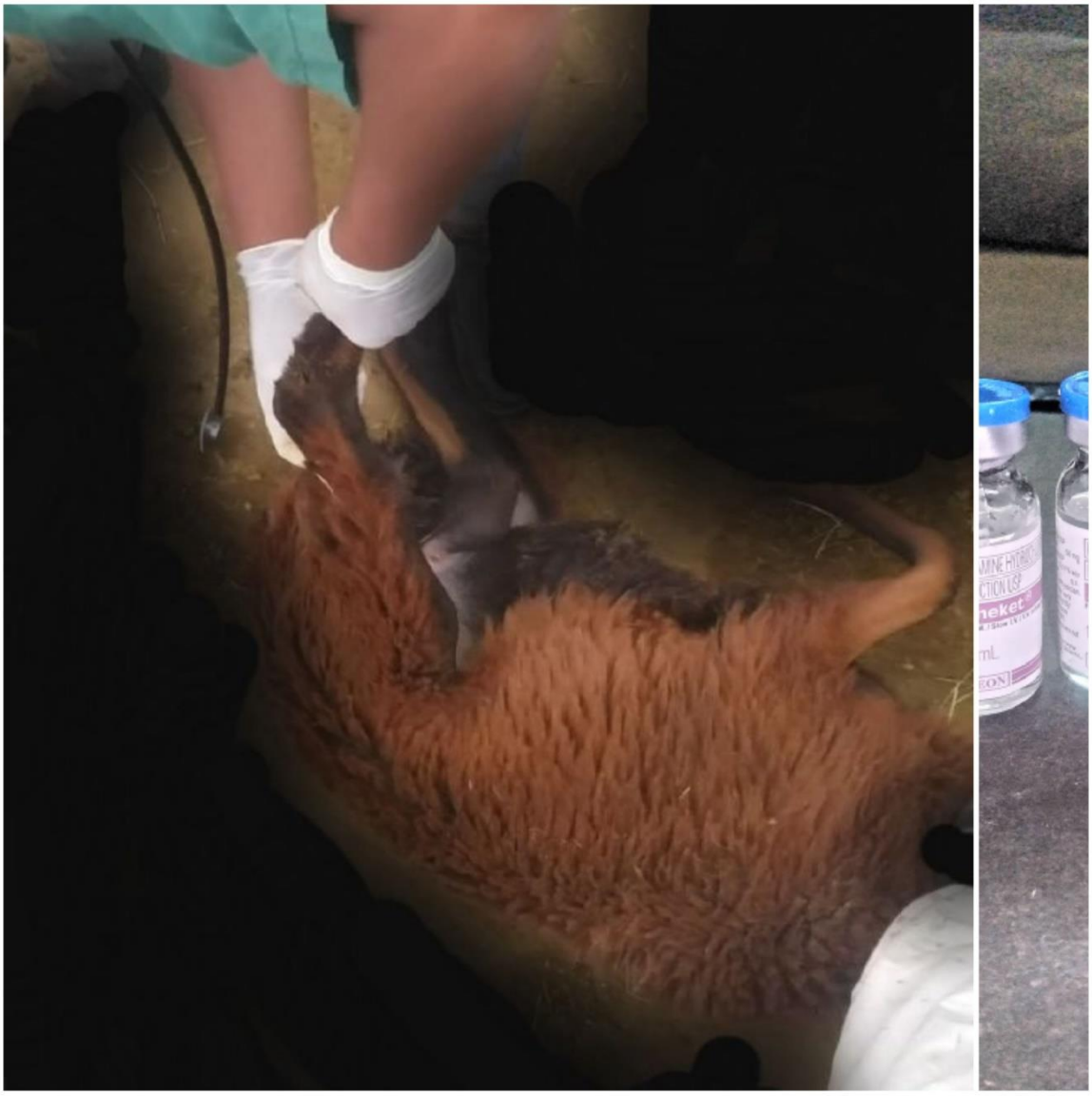
Name: Dr Guesh Negash

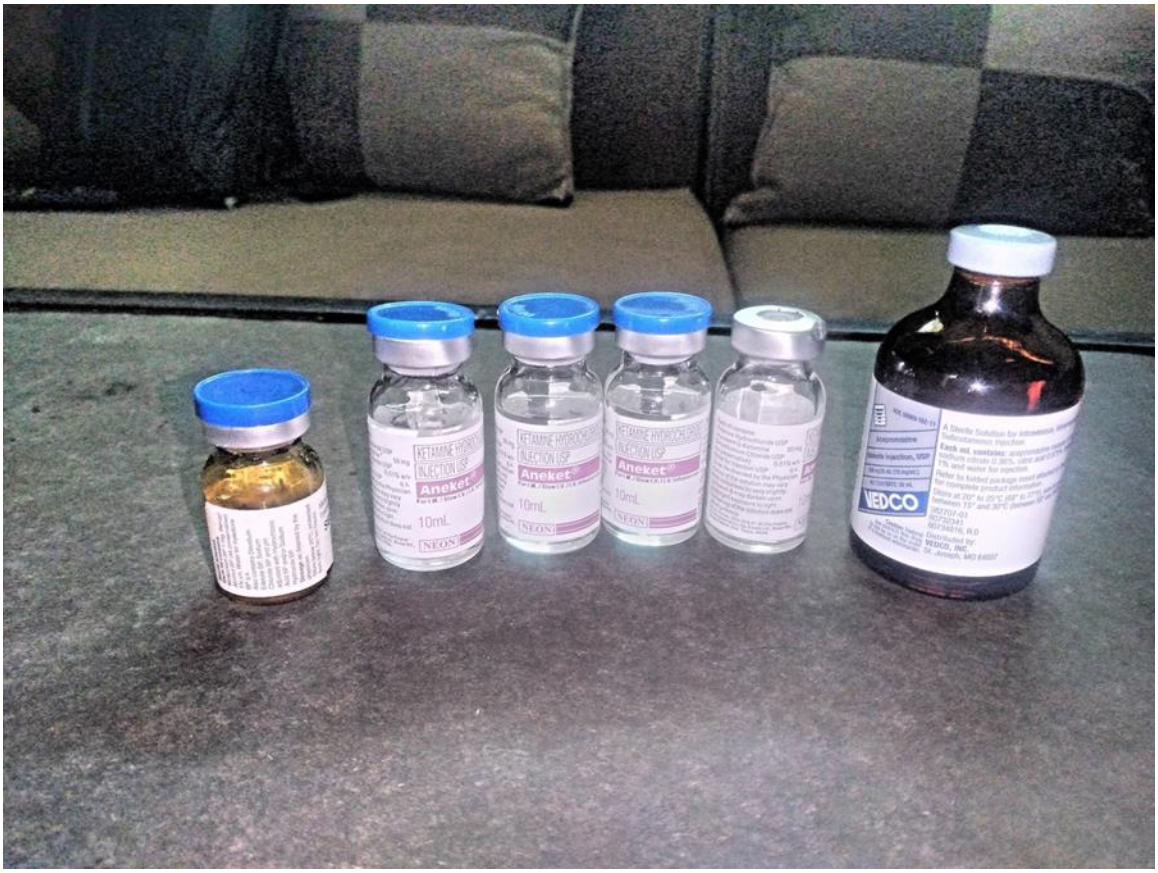
Signature: for

Abrha Bsrat (DVM)
Dean

Tel No. +251-3-44-40-16-10 P. O. Box 2084, Mekelle, Tigrai, Ethiopia

III. Experimental animal preparation




Aneket
10mL
NEON
VEDCO