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Comparison of Physiological Effects of Two Anesthetic Protocols in Rabbits Undergoing Orthopedic Surgery

[1]Mana, H. P., [2]Bada, A. A., [2]Sani, M. A., [3]Yusuf, Z. B., [2]Ochube, G. E., [2]Bappah, M. N., [4]Emmanuel, E. G., [5]Adamu, S. [2]Hassan, A. Z.

[1]Veterinary teaching Hospital, University of Maiduguri, Borno State
[2]Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine Ahmadu Bello University Zaria
[3]Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, University of Maiduguri
[4]Veterinary Teaching Hospital, Ahmadu Bello University Zaria
[5]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria

[*]*Author for Correspondence: sekundayo90@yahoo.com*

ABSTRACT

Rabbits present unique anesthetic challenges due to their high metabolic rate, stress susceptibility, and increased perioperative mortality risk. The physiological effects of two injectable anesthetic protocols Midazolam-Ketamine (MK) and Xylazine-Ketamine (XK) in rabbits undergoing orthopedic Surgery (implant removal) are compared in this study. A total of six rabbits were randomly assigned to two groups (n=3 per group) to receive either MK (5 mg/kg midazolam + 20 mg/kg ketamine) or XK (5 mg/kg xylazine + 20 mg/kg ketamine) intramuscularly. At baseline and at 0, 30, 60, 90, and 120 minutes after induction, core temperature, respiratory rate, and pulse rate were measured. The results showed that the XK combination caused severe respiratory depression immediately after induction (mean difference: −23.33 breaths/min, 95% CI: −25.31 to −21.35) and considerable hypothermia, with a mean temperature difference of −3.37°C after 60 minutes (95% CI: −4.51 to −2.22). While the Midazolam-Ketamine (MK) combination provided better physiological stability in maintaining normothermia and respiratory function, the Xylazine-Ketamine (XK) protocol offered more effective postoperative analgesia despite causing significant hypothermia and respiratory depression. These findings suggest MK may be a safer option for routine use, whereas XK may be considered when analgesia is prioritized and adequate supportive care is ensured. Furthermore, in resource-limited circumstances, Xylazine-Ketamine remains a feasible alternative when complemented with active warming and respiratory monitoring.

Keywords: Anaesthesia; Hypothermia; Midazolam-Ketamine; Physiological Parameters; Rabbit; Xylazine-Ketamine

INTRODUCTION

After dogs and cats, rabbits are the third most commonly anesthetized species in veterinary practice (Brodbelt *et al*., 2008). Despite their prevalence as patients, they present a significant anesthetic challenge due to a combination of physiological and anatomical factors that render them high-risk patients (Mohammed and Abd-Alhafid, 2023; Schmid *et al*., 2025). Compared to larger companion animals, rabbits possess a limited physiological reserve, which contributes to their considerably higher risk of morbidity and mortality associated with general anesthesia. The documented anesthesia-related mortality rate in rabbits is 0.73%, which is substantially greater than that of dogs (0.05%) and cats (0.11%) (Brodbelt, 2009). Approximately 40% of these peri-anesthetic deaths are attributed to cardiorespiratory problems, though the specific cause often remains unknown (Brodbelt, 2009). This elevated risk is further compounded by their small body size and high surface-area-to-volume ratio, which makes them highly susceptible to hypothermia, as well as their pronounced stress response, which can complicate pre-anesthetic handling and induction (Johnson-Delaney and Orosz, 2011; Wenger, 2012; Fusco *et al*., 2021).

The development of safe and efficient anesthetic protocols is therefore crucial for veterinarians performing procedures in this species (Gardhouse and Sanchez, 2022). While inhalation induction is possible, the use of a face mask is often exceedingly stressful for rabbits (Johnson-Delaney and Orosz, 2011; Wenger, 2012). Consequently, injectable anesthetic protocols remain a cornerstone of rabbit anesthesia (Dandea *et al*., 2025). To enhance muscle relaxation and analgesia beyond that provided by ketamine alone, it is frequently combined with sedatives such as xylazine or benzodiazepines like midazolam (Lee *et al.,* 2010). However, each adjunct carries a distinct physiological profile. Xylazine, a potent alpha-2 adrenergic agonist, provides reliable sedation and muscular relaxation but is known to cause dose-dependent cardiovascular depression, bradycardia, and peripheral vasodilation that can lead to hypothermia (Marín *et al*., 2020). In contrast, midazolam provides sedation and

amnesia with a reportedly higher margin of safety for cardiorespiratory function (Wenger, 2012).

While both combinations are used clinically, direct comparisons of their physiological effects in rabbits undergoing surgical procedures are limited. Specifically, there is a gap in the literature regarding their comparative impact on thermoregulation and respiratory function in the context of orthopedic surgery. To address this gap, we designed a prospective study to objectively compare the physiological effects of xylazine-ketamine and midazolam-ketamine combinations in rabbits undergoing orthopedic surgery.

We hypothesized that the midazolam-ketamine combination would result in superior physiological stability specifically, better preservation of normothermia and respiratory function compared to the xylazine-ketamine combination. Conversely, we anticipated that the xylazine-ketamine protocol might provide more effective analgesia, albeit with a higher incidence of adverse physiological effects. Through analyzing core body temperature, respiratory rate, and pulse rate, and by using 95% confidence intervals to estimate the magnitude and precision of the observed effects, this study aims to identify the protocol that provides superior physiological stability and an improved safety profile for anesthetic management in this high-risk species.

MATERIALS AND METHOD

Experimental Animals

A total of six (6) adult rabbits scheduled for aseptic removal of orthopedic bone plates were used in this study. The animals were housed in the experimental animal unit of the institution under standard conditions with a controlled light-dark cycle and provided with a standard diet and water ad libitum. Guided by the 3Rs (Reduction, Refinement, Replacement) framework for ethical animal research, the sample size was limited to the minimum (three animals per group) necessary to yield meaningful scientific data and compare the effects of the two anesthetic protocols.

Ethical Statement

All experimental procedures involving animals were reviewed and approved by the Ahmadu Bello University Committee on animal use and care (ABUCAUC) with the reference number ABUCAUC/2025/049 prior to the commencement of the study. The study strictly followed the ARRIVE 2.0 guidelines to ensure comprehensive reporting, reproducibility, and ethical rigor. Every procedure was carried out by our team consisting of trained veterinarians utilizing methods intended to minimize pain, suffering, and distress. The animals were later reintegrated back to the community for reproduction.

Experimental Design and Protocols

The rabbits were randomly assigned to either the control or treatment group (n=3 per group). This was achieved by flipping a fair coin for each animal; 'heads' assigned the rabbit to one group, and 'tails' to the other. The anaesthetic protocols were administered intramuscularly (IM) as follows: The first group received a combination of Midazolam (Zolamid I.V./I.M./Rektal Kullanim Icin Solution Iceren Ampul 15 Mg/3Ml Defarma Turkey) at (5 mg/kg) and Ketamine (10mg/mL, 20mL Vial | Bound tree 000 Bradenton Ave, Dublin, OH 43017 USA) at (20 mg/kg). The second received a combination of Xylazine (5 mg/kg) and Ketamine (20 mg/kg).

Data Collection

Baseline physiological parameters, including rectal temperature (°C), respiratory rate (breaths/min), and pulse rate (beats/min), were recorded for all animals prior to drug administration. The time of onset of anaesthesia, defined as the loss of the righting reflex, was noted immediately after the injection of the anaesthetic agents. The physiological parameters (rectal temperature, respiratory rate, and pulse rate) were subsequently re-recorded at the following time points: immediately at onset (0 minutes), and at 30-, 60-, 90-, and 120-minutes post-induction.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). The effects of Time, Group, and their Interaction on physiological parameters were analyzed using a two-way repeated measures ANOVA. The mean difference (MD) between the MK and XK groups at each time point was calculated, along with its 95% Confidence Interval (CI). A 95% CI that does not cross zero was considered to indicate a statistically significant difference at the α=0.05 level. The onset time between the two groups was compared using an unpaired t-test, reported as MD with 95% CI. All statistical analyses were performed using GraphPad Prism software, version 9.0

Surgical Removal of the Bone Plates

Pre-Surgical Imaging and Planning:

Prior to surgery, a standard diagnostic X-ray was taken of the affected limb to confirm the precise location, size, and condition of the bone plate and screws, allowing for accurate surgical planning and incision placement as seen in figure 1 below.

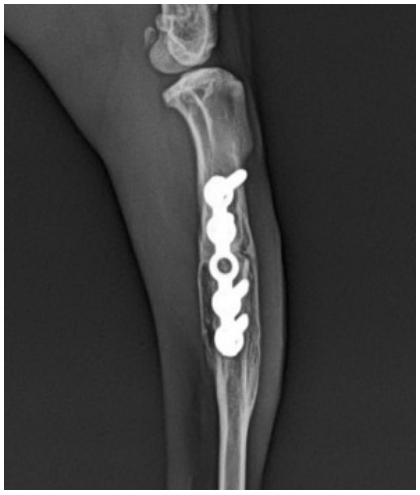

Figure 1: Preoperative radiograph locating the bone plate

Surgical Preparation

The rabbit was placed under general anesthesia using one of the two intramuscular protocols (Xylazine-Ketamine or Midazolam-Ketamine). The surgical site was then shaved, aseptically scrubbed with a 0.3 % chlorhexidine followed by 10% povidone-iodine solution, and draped to maintain a sterile field throughout the procedure. Anesthesia was maintained throughout the procedure using the first

injectable combination, with physiological parameters (temperature, respiration rate, and pulse rate) continually monitored at certain intervals (0, 30, 60, 90, and 120 minutes) to assess stability under each regimen. A longitudinal incision was made over the previous surgical scar to reveal the bone plate. Each screw was carefully removed using a suitable screwdriver, and the plate was gradually elevated and retrieved from the bone surface while minimizing stress to the underlying bone and periosteum, as illustrated in Figure 2. After removing the plate, the surgical site was irrigated with sterile saline. The fascial and subcutaneous layers were closed with absorbable sutures (Chromic catgut size 2.0), and the skin was sutured with nylon size 2.0. Surgery duration averaged 30 ± 2 minutes across all rabbits and was performed by a single surgeon with an assistant and a flunky. Postoperative analgesia (piroxicam) was administered five (%) after Surgery for all the groups at 3mg/kg once daily for 3 days. The Rabbits were monitored during recovery until full ambulatory.

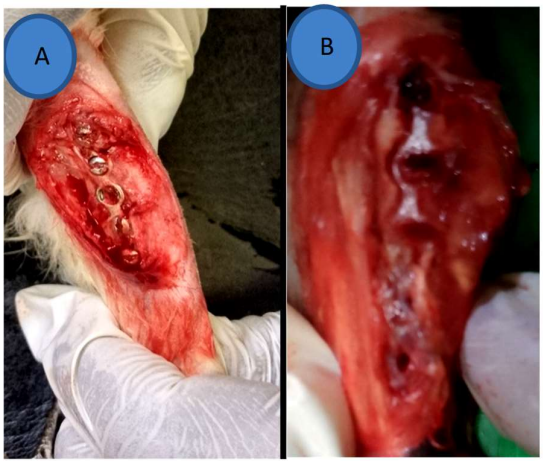

Figure 2: (A) Excised skin and exposed bone plate; (B) Bone plate being gently removed while preserving surrounding tissues.

RESULTS

The mean onset time was 3.33 ± 0.58 minutes for the Midazolam-Ketamine (MK) group and 4.00 ± 1.00 minutes for the Xylazine-Ketamine (XK) group. The mean difference was -0.67 minutes (95% CI: -2.68 to 1.35). The negative value indicates a faster onset in the MK group; however, the confidence interval crosses zero, indicating that this observed difference is not statistically significant (Table 1).

Furthermore, a significant Group × Time interaction effect on core body temperature was observed ($p < 0.005$). As shown in Table 2, the Xylazine-Ketamine (XK) group experienced a pronounced and progressive decline in temperature following anesthetic induction. By 60 minutes post-induction, the mean rectal temperature in the XK group had dropped to 34.9 ± 0.6 °C, while the Midazolam-Ketamine (MK) group maintained stable normothermia at 38.3 ± 0.1 °C. This resulted in a substantial mean difference of 3.37 °C (95% CI: 2.22 to 4.51), with the confidence interval not crossing zero, indicating a statistically significant and clinically critical difference.

Although the XK group's temperature showed gradual recovery from the 90-minute mark onward, it remained significantly lower than that of the MK group at all post-induction time points through 90 minutes, as indicated by 95% confidence intervals that did not cross zero. By 120 minutes, the confidence interval crossed zero, suggesting the temperatures were no longer statistically different between groups. Additionally, A significant Group × Time interaction was found for respiratory rate ($p < 0.005$). At baseline, both groups exhibited similar rates. Immediately following anesthetic induction (0 minutes), the respiratory rate in the Xylazine-Ketamine (XK) group dropped markedly to 35.7 ± 0.6 breaths/min, while the Midazolam-Ketamine (MK) group remained stable at 59.0 ± 1.0 breaths/min. This resulted in a mean difference of 23.33 breaths/min (95% CI: 21.35 to 25.31), indicating a statistically significant and clinically severe respiratory depression in the XK group.

This severe respiratory depression persisted at 30 and 60 minutes. Although the XK group's respiratory rate showed gradual recovery beginning at 90 minutes, it did not return to a level statistically equivalent to the MK group until the 120-minute time point, at which the confidence interval for the mean difference crossed zero (Table 3).

Furthermore, table 4 shows no significant Group × Time interaction was found for pulse rate ($p = 0.89$). At baseline, the Xylazine-Ketamine (XK) group recorded a pulse rate of 162.0 ± 4.4 beats/min, while the Midazolam-Ketamine (MK) group recorded 158.3 ± 3.2 beats/min, resulting in a mean difference of -3.67 beats/min (95% CI: -13.90 to 6.57). Throughout the monitoring period, pulse rates in both groups remained stable and closely aligned, with all subsequent between-group mean differences being less than 1.33 beats/min and all 95% confidence intervals crossing zero. For example, at 120 minutes, the XK group recorded 156.7 ± 0.6 beats/min and the MK group recorded 157.7 ± 2.1 beats/min, with a mean difference of 1.00 beats/min (95% CI: -2.33 to 4.33).

Table 1: Onset of Anaesthesia (Loss of Righting Reflex)

Group	Mean Onset Time (min) ± SD	Mean Difference (min)	95% Confidence Interval of Difference
Midazolam-Ketamine (MK)	3.33 ± 0.58	-0.67	-2.68 to 1.35
Xylazine-Ketamine (XK)	4.00 ± 1.00		

*Note: A negative mean difference indicates a faster onset for the MK group. The confidence interval crossing zero indicates that the difference is not statistically significant.

Table 2: Mean Rectal Temperature (°C) and Between Group

Time Point	Group XK (n=3)	Group MK (n=3)	Mean Difference (A - B)	95% CI of Difference
Baseline	37.3 ± 0.4	37.7 ± 0.5	0.40	(-1.06 to 1.86)
0 min	35.7 ± 0.7	38.3 ± 0.4	2.63	(1.47 to 3.79)
30 min	35.7 ± 0.7	38.3 ± 0.4	2.63	(1.47 to 3.79)
60 min	34.9 ± 0.6	38.3 ± 0.3	3.37	(2.22 to 4.51)
90 min	36.1 ± 0.6	37.8 ± 0.0	1.70	(0.55 to 2.85)
120 min	37.1 ± 0.0	38.0 ± 0.3	0.93	(0.04 to 1.82)

*Note: A negative mean difference indicates a lower temperature in the XK group compared to the MK group.

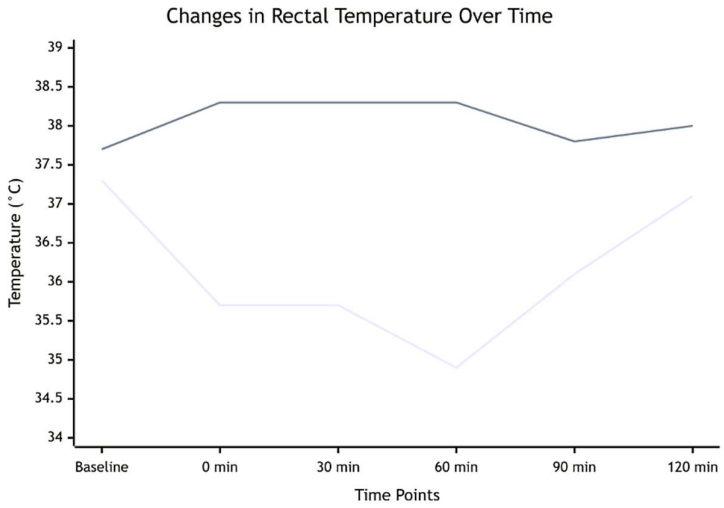

Figure 2: A line graph showing two lines: XK line drops sharply; MK line remains stable

Table 3: Mean Respiratory Rate (breaths/min) and Between-Group Difference

Time Point	Group XK (n=3)	Group MK (n=3)	Mean Difference (MK - XK)	95% CI of Difference
Baseline	58.7 ± 1.5	59.7 ± 0.6	1.00	(-1.- 36 to 3.36)
0 min	35.7 ± 0.6	59.0 ± 1.0	23.33	(21.35 to 25.31)
30 min	36.3 ± 0.6	58.7 ± 0.6	22.33	(20.35 to 24.31)
60 min	39.3 ± 1.2	59.0 ± 1.0	19.67	(17.12 to 22.21)
90 min	47.7 ± 1.5	60.3 ± 1.5	12.67	(10.12 to 15.21)
120 min	59.7 ± 1.5	60.7 ± 0.6	1.00	(-1.36 to 3.36)

*Note: A positive mean difference indicates a higher respiratory rate in the MK group compared to the XK group.

Table 4: Mean Pulse Rate (beats/min) and Between-Group Differences

Time Point	Group XK (n=3)	Group MK (n=3)	Mean-Difference (MK - XK)	95% CI of Difference
Baseline	162.0 ± 4.4	158.3 ± 3.2	-3.67	(-13.90 to 6.57)
0 min	155.3 ± 2.5	156.7 ± 2.1	1.33	(-2.74 to 5.41)
30 min	154.3 ± 3.8	155.0 ± 2.0	0.67	(-4.93 to 6.26)
60 min	157.7 ± 2.1	159.0 ± 3.6	1.33	(-4.49 to 7.16)
90 min	156.7 ± 2.1	157.7 ± 1.2	1.00	(-2.68 to 4.68)
120 min	156.7 ± 0.6	157.7 ± 2.1	1.00	(-2.33 to 4.33)

* Data presented as mean ± standard deviation (SD). No statistically significant differences between groups at any time point ($p > 0.05$ for all).

DISCUSSION

This study used 95% confidence intervals (CIs) to compare the effects of two anesthetic regimes in rabbits. The most clinically relevant observation was the extreme hypothermia caused by the Xylazine-Ketamine (XK) combination. This is consistent with the known physiological effects of alpha-2 agonists such as xylazine, which produce peripheral vasodilation and greatly impair thermoregulation (Giovanitti *et al*., 2015). (Hobbs *et al*., 1991) previously emphasized the difficulty of xylazine-induced hypothermia, discovering that combinations comprising xylazine and ketamine were particularly related with significant reductions in body temperature.

The findings of this investigation show significant differences in the pharmacological profiles of the two anesthetic regimens. The Midazolam-Ketamine (MK) group had a faster onset of anesthesia (3.33 ± 0.58 minutes) than the Xylazine-Ketamine (XK) group (4.00 ± 1.00 minutes), with a mean difference of -0.67 minutes. However, the 95% confidence interval for this difference (-2.68 to 1.35) crossed zero, implying that the observed speedier onset was not statistically significant. There were more clinically meaningful differences in the quality and duration of postoperative analgesia. The choice of anesthetic considerably altered body temperature, as demonstrated by a significant Group x Time interaction ($p < 0.005$), highlighting the varied physiological implications of these medication combinations. Furthermore, our data demonstrate that the xylazine-Ketamine (XK) group maintained a significantly lower core body temperature throughout the observation period. The magnitude of this difference was significant at the 60-minute mark; we were 95% confident that the true mean temperature under the XK protocol was lower than under the midazolam-Ketamine protocol. This large and precise effect indicates a serious risk associated with xylazine that can compromise recovery and may lead to shock.

A similarly clear and dramatic effect was observed in respiratory rates. The 95% CI at time 0 minutes (21.35 to 25.31 breaths/min) indicates a severe depressive effect of the XK combination immediately post-induction. The gradual narrowing of this difference over time, with the CI finally crossing zero at 120 minutes, visually demonstrates the recovery process. In contrast, the MK regimen exhibited stable respiratory rates, indicating a negligible depressive effect on respiration. This finding is supported by Mahmood (2022), who also reported that a ketamine-xylazine combination led to a significant reduction in respiratory rate. The respiratory depression from xylazine is attributed to its action on central alpha-2 adrenoceptors, which reduces catecholamine release and central nervous system activity (Flecknell, 1998; Fitri *et al*., 2021)

From a cardiovascular standpoint, the pulse rate analysis reinforces the relative safety of both regimens in our study. All CIs for pulse rate differences were narrow and crossed zero, indicating that any observed variations were small and consistent with random chance rather than a true, clinically relevant drug effect. This is consistent with the findings of Mahmood (2022), who reported no significant adverse effects on heart rate with a low-dose ketamine-midazolam-xylazine combination, suggesting that careful dosing can achieve cardiovascular stability (Fitri *et al*., 2021).

Furthermore, a notable finding was the superior postoperative analgesia provided by the Xylazine-Ketamine (XK) combination compared to Midazolam-Ketamine (MK).

Limitations

This study has several limitations that should be considered when interpreting the results. First, the sample size was small (n=3 per group). While the study was sufficiently powered to detect large physiological effects, such as the observed differences in temperature and respiratory rate, it may not have been able to detect smaller, yet clinically relevant, differences in other parameters like pulse rate. A larger sample size would provide more precise estimates of the treatment effects and increase the generalizability of the findings. Second, while the study focused on physiological parameters, it did not include a validated, objective assessment of analgesia. A formal pain scoring system would be valuable in future studies to directly compare the analgesic efficacy of these two protocols. Third, the follow-up period was limited to 120 minutes post-induction; longer-term monitoring of recovery parameters was not performed. Finally, all surgeries were performed by a single surgical team, which enhances internal consistency but may limit the applicability of the results to other settings.

Conclusion

This study suggests that the Midazolam-Ketamine (MK) combination may offer physiological advantages in terms of temperature and respiratory stability, while the Xylazine-Ketamine (XK) protocol appears to provide superior analgesia. These findings, though limited by sample size, indicate that protocol selection should consider both patient stability and analgesic requirements, with appropriate supportive care when XK is used. The XK protocol may therefore remain a viable option, particularly in resource-limited settings where midazolam is unavailable or cost-prohibitive, provided that clinicians implement supportive care such as active warming and respiratory monitoring to mitigate its documented adverse effects. Further research with larger sample sizes is warranted to confirm these findings and to directly compare the analgesic properties of these two protocols.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contribution

HPM conceptualized the study, performed the surgeries, and wrote the draft manuscript. AAB, MAS, and EGE assisted with surgeries, monitored anesthesia, and collected physiological data. ZBY and MNB contributed to data analysis and manuscript preparation. GEO and AZH supervised the surgical procedures and provided clinical oversight. SA contributed to the helped in edition, and approved the final manuscript.

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