The Atipamezole Plays a Great Role in the Quick Recovery of the Male Rabbit Anesthetized for Neutering

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ABSTRACT

Delay in recovery from general anesthesia is always hazardous to life. Therefore, this study aims to evaluate the effect of atipamezole in the recovery of rabbits anesthetized with xylazine-ketamine undergoing neutering. Ten male rabbits were randomly assigned to two groups (n = 5 per group), where group XK was treated with xylazine (5 mg/kg bwt) and ketamine (30 mg/kg bwt) intramuscularly without atipamezole, and group XKA was treated with xylazine (5 mg/kg bwt), ketamine (30 mg/kg bwt), and atipamezole (0.5mg/kg bwt) intramuscularly. Atipamezole was administered intramuscularly 30 minutes post-injection of the anesthetic combination. Physiological parameters, induction time, duration of anesthesia, recovery time, hematological, and biochemical parameters were measured. In group XKA, heart rate, temperature, and respiration that decreased during anesthesia, reached almost normalcy during recovery, rather than group XK. Significant differences in induction, duration of anesthesia, and recovery periods were observed between the two groups. Group XKA showed a short recovery time compared to Group XK. The duration of anesthesia was longer in group XK in comparison to group XKA. The recovery period was short in group XKA due to the atipamezole. There was no effect of atipamezole on healing. There were no significant differences in hematological parameters between the two groups. Taken all together, we conclude that the atipamezole has a great role in the reduction of recovery time of the rabbit with xylazine-ketamine induced anesthesia without significant adverse effects in clinical and blood parameters during neutering.

Keywords: Recovery, Atipamezole, Xylazine-ketamine, Male rabbits, Neutering

INTRODUCTION

Rabbits are extensively utilized as experimental models in biomedical research owing to their compact body size, manageable temperament, and the wide availability of organs and tissues suitable for scientific analysis. The domestic rabbit is also valued as a source of low-cholesterol animal protein and has recently gained popularity as a household pet. Consequently, veterinarians and researchers frequently manage rabbits requiring diagnostic, therapeutic, or surgical interventions that demand appropriate anesthetic management (Hassan et al., 2024).

In the present research environment, anesthetic agents constitute the most commonly used class of drugs for the control of pain, particularly the pain associated with surgical interventions. In rabbits, neutering (ovariectomy/ovariohysterectomy in females and orchiectomy in males) is one of the most frequently performed surgical procedures in veterinary practice and biomedical research. The major indications for neutering in female rabbits include reproductive tract disorders such as uterine adenocarcinoma, pyometra,

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mammary neoplasia, uterine neoplasia, and endometrial hyperplasia. Additional indications involve the reduction of pregnancy- and parturition-related complications such as metritis, mastitis, dystocia, metrorrhagia, and glandular cystic uterine hyperplasia with secondary infections leading to chronic uterine inflammation. In male rabbits, neutering is indicated for population control, prevention of testicular disease, and reduction of sexually aggressive and marking behaviors (Coulter et al., 2011).

The procedure should be undertaken only when the rabbit is in a physiologically stable condition and fit to withstand general anesthesia. However, certain contraindications exist, including hypothermia, dehydration, severe systemic illness, and excessive mydriasis, which may increase anesthetic risk (Raillard et al., 2019).

Among injectable anesthetic protocols, the xylazine–ketamine combination is one of the most frequently applied regimens in rabbits due to its reliability, cost-effectiveness, and ease of administration. Xylazine is an alpha-2 adrenergic agonist that produces sedation, muscle relaxation, and analgesia by inhibiting the release of norepinephrine at presynaptic nerve terminals in the central nervous system. Ketamine is a dissociative anesthetic agent that induces profound analgesia and amnesia by blocking N-methyl-D-aspartate (NMDA) receptors. When used together, xylazine and ketamine produce balanced anesthesia characterized by sedation, analgesia, muscle relaxation, and sufficient surgical depth, making this combination highly suitable for rabbit neutering surgeries (Pennasilico et al., 2025).

However, xylazine is associated with certain adverse effects, such as bradycardia, hypotension, respiratory depression, and hypothermia, while ketamine may cause muscle rigidity and prolonged recovery when used alone. The duration of surgical anesthesia with xylazine–ketamine is generally limited, and recovery may be delayed in some animals (Irwin et al., 2023).

To overcome these limitations, atipamezole, a highly selective alpha-2 adrenergic receptor antagonist, is used for the rapid and effective reversal of xylazine-induced sedation and cardiovascular depression. Atipamezole competitively blocks alpha-2 receptors and restores the release of norepinephrine, thereby improving heart rate, respiratory function, alertness, and thermoregulation. The administration of atipamezole significantly shortens recovery time and enhances postoperative safety and comfort in rabbits undergoing neutering (Coulter et al., 2011).

The xylazine–ketamine–atipamezole anesthetic protocol, therefore, offers several advantages, including reliable surgical anesthesia, good muscle relaxation and analgesia, controlled and smooth induction, rapid and safe recovery with atipamezole reversal, and reduced perioperative stress and mortality. Little research has been conducted, but the role of atipamezole in the recovery is still not clear, especially in the rabbit during surgery. That’s why the study on the role of atipamezole is important to get clear information. We hypothesize that atipamezole has the ability to reduce the recovery time in the rabbits during surgery. Therefore, the objective of this study is to assess the contribution of atipamezole in the recovery of rabbits anesthetized with xylazine–ketamine–induced undergoing neutering.

MATERIALS AND METHODS

Animals and Experimental Design

Our present study was designed to include 10 apparently healthy male rabbits weighing 1.31±0.1 kg and aged 10±2 months, purchased from the Bonder Bazar, Sylhet, Bangladesh. The research was conducted at the Laboratory of the Department of Surgery and Theriogenology and at Professor Moshleh Uddin Ahmed Chowdhury Veterinary Teaching Hospital of Sylhet Agricultural University. The duration of the research work was allocated from December 2024 to November 2025. The rabbits were not required to be fasted overnight before induction of anesthesia. The rabbits were examined clinically to detect any pathological condition. Before starting the experiment, the body weight of each rabbit was taken using a weighing machine. The rabbit was then placed on the Operation Table in the O.T. of Professor Moshleh Uddin Ahmed Chowdhury Veterinary Teaching Hospital of Sylhet Agricultural University. The anesthesia was always performed in the morning throughout the course of the investigation. The area of injection was sterilized with 70% alcohol. The anesthetic agent was then administered into the muscle at the dose indicated in the experimental design. The

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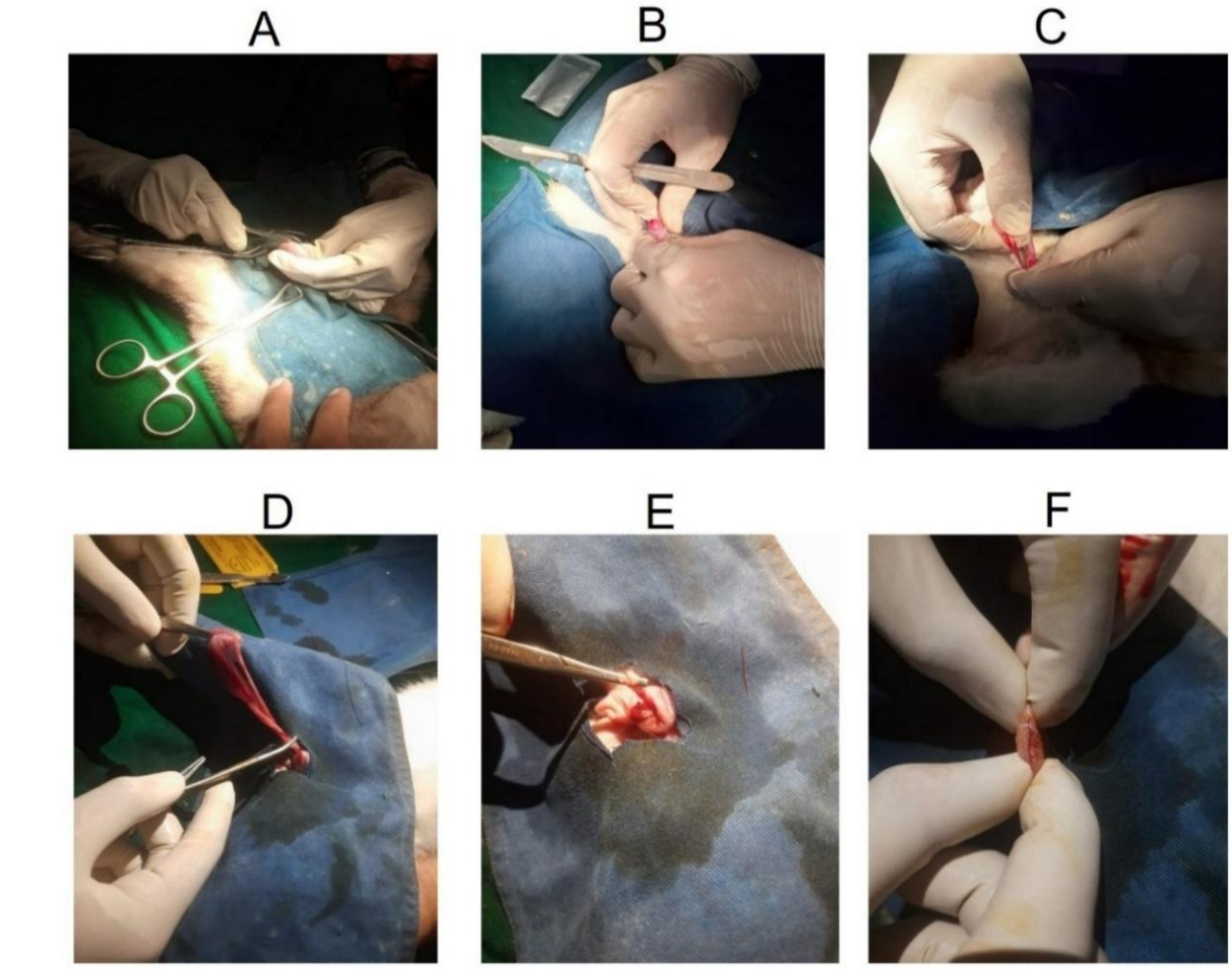
agents were administered in the thigh muscle using an insulin syringe. All drugs were administered consecutively in separate syringes. The experimental rabbits were randomly divided into two (2) groups, each group containing five (5) rabbits. One was the xylazine hydrochloride- ketamine hydrochloride (XK) group, serving as the control, where rabbits were injected with the xylazine (5 mg/kg body weight) and ketamine hydrochloride (30mg/kg body weight) combination (Supplementary figure 1) intramuscularly for neutering (Figure 1) and the other was the xylazine hydrochloride- ketamine hydrochloride and atipamezole (XKA) group, serving as the treatment, where rabbits were injected with the xylazine (5 mg/kg body weight) and ketamine hydrochloride (30mg/kg body weight) combination intramuscularly for neutering (Figure 1) and during 30 minutes of anesthesia atipamezole hydrochloride (0.5mg/kg body weight) (Supplementary figure 1) was injected intramuscularly. Before the operation, the site was shaped, draped, and prepared with povidone-iodine.

The sample size (n=5 per group) was determined based on a power analysis assuming a large effect size (Cohen's d = 1.5) for the primary outcome (recovery time), with $\alpha = 0.05$ and power = 0.80. A post-hoc power analysis using the observed difference in recovery times (mean difference = 67.0 min, pooled SD = 7.7 min) yielded a Cohen's d of 8.7 and power > 0.99, indicating that the study was adequately powered to detect the large effect observed. However, the small sample size limits the ability to detect smaller but potentially clinically meaningful differences in secondary outcomes (e.g., hematological parameters). Future studies with larger sample sizes are needed to confirm these findings.

Figure 1. The procedure of neutering step by step. A) making the incision on the tip of the testicle of the rabbit, B) pressing the scrotum to remove the testicle, C) exteriorizing the spermatic cord, including the testicle from the inside, D) ligation and excision of the testicle, E) crushing the cutting end of the spermatic cord, and F) bringing the cutting edge in apposition without suturing.



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Ethical Statements

The authors confirm that the experiments were performed according to the standard animal welfare guidelines with permission from the Animal Ethics Committee of Sylhet Agricultural University (Approval ID. #ARP2026099).

Materials

We purchased xylazine (Xyla, each ml contains xylazine hydrochloride 23.32 mg equivalent to 20 mg, Interchemie Werken Ltd, Holland, 50ml vial), ketamine (G-ketamine, each ml contains ketamine hydrochloride BP 50mg, Gonoshasthaya Pharmaceuticals Ltd, Bangladesh, 10ml vial) and atipamezole (Atipamezole hydrochloride, each ml contains Atipamezole Hydrochloride 5mg, lot no. SL3IJ-AL, Tokyo Chemical Industry Co. Ltd, 25mg) to use in the experiments. A thermometer and a stethoscope were used to observe the clinical parameters. A digital balance was used to weigh the body weight. We also used syringes (1ml, 3ml), a 27G butterfly needle, a timer, a pen, a scale, a camera, a record book, etc. For blood collection, we used red and purple tubes and a tube rack. For the surgical procedure, we used gloves, masks, surgical gauze, cotton, OT dress, surgical blade, BP handle, trimer, shaving Blade, soap, Chlorhexidine, alcohol pad, povidone iodine, draping towel, towel clamp, Allis tissue forceps, hemostatic forceps, needle holder, Catgut- 4/0, scissors, etc. Antibiotics, antihistamines, and NSAIDs were used after neutering and in post-operative care.

Period of Induction, Duration of Anesthesia, and Recovery from Anesthesia

The period extending from the time of injection up to the lateral recumbency with the reduction of different reflexes was mentioned as the period of induction. The duration of anesthesia starts from the onset of anesthesia up to painless surgery. The recovery period extends from the stage of reappearance of consciousness up to the stage when the rabbits were in full sense and response to external stimuli.

The recovery period was defined as the time from the end of surgery (completion of neutering) to the return of the righting reflex (the ability of the rabbit to right itself from lateral recumbency to sternal recumbency) and the ability to stand unaided. The observer assessing recovery times was blinded to group allocation (i.e., unaware whether atipamezole had been administered).

Determination of Clinical Parameters

Respiration rate (per minute), heart rate (per minute), and rectal temperature (°F) of the experimental rabbits were recorded before the anesthesia and during the course of the anesthetic period at 15 minutes, 30 minutes, and 45 minutes (Supplementary figure 3).

Respiration Rate

The respiration rate was recorded by counting respiration visually, i.e., by watching and counting the movements of the abdomen. Care was taken not to excite the rabbits before and during monitoring.

Heart Rate

Heart rate was determined using a stethoscope. For this, the diaphragm of the stethoscope was placed over the chest region of the rabbits.

Rectal Temperature

The rectal temperature was taken by inserting a clinical thermometer into the rectum. The bulb of the thermometer was moistened with Vaseline before insertion into the rectum. The position of the thermometer in the rectum was such that the bulb of the thermometer touched the rectal mucosa. The thermometer was held in position for 90 seconds.

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Blood Sample Collection

For each rabbit, a blood sample was collected before administration of injectable anesthetic drugs. (pre-anesthetic sample), 45 minutes post-anesthesia and after recovery following completion of neutering under anesthetic agents (post-anesthetic sample). For the sex hormonal test, the blood was collected before anesthesia and after 7 days of neutering. Each blood sample was collected from the marginal ear vein using an insulin syringe or butterfly needle attached to a plastic syringe (Supplementary figure 3). Blood was immediately transferred into an ethylenediaminetetraacetic acid (EDTA) micro-collection tube. Pre-anesthetic samples and post-anesthetic samples were collected in all 10 rabbits.

Reflexes Monitored

The depth of anesthesia was monitored by measuring the withdrawal reflex, palpebral reflex, corneal reflex, and pedal reflex.

Other Observations

Several miscellaneous parameters like defecation, urination, salivation, and lacrimation were observed carefully during each experiment.

Hematological Analysis

A complete blood count (CBC) was performed on each blood sample on the day of blood collection at the Laboratory of the Department of Surgery and Theriogenology of Sylhet Agricultural University using an automated hematology analyzer (Mythic-18 Automated Hematology Analyzer). Hematological values provided by the hematology analyzer included: Red blood cell (RBC) count; hemoglobin (Hb) concentration; hematocrit (HCT); mean red blood cell volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red blood cell distribution width (RDW); total white blood cell (WBC) count, segmented Neutrophils (SEG) count; Eosinophils (EOS) count, Basophils (BASO) count; Lymphocyte (LYMPHO) count; Monocytes (MONO) count; and platelet (PLT) count. The parameters were counted as monocytes in percentage, Eosinophils in percentage, Basophils in percentage, MCV (fl) mean corpuscular volume in femtoliters, HB(gm/dl)- Hemoglobin in gram per deciliter, RBC(mil/ul) Red Blood Cell in million per micro liter, WBC(/cumm)- White Blood Cell in per cubic millimeter, Platelets(mil/mm)- Platelets in million per milliliters, Neutrophils in Percentage, RDW- red cell distribution width in percentages, MCHC mean corpuscular hemoglobin concentration in gram per deciliters, HCT- hematocrit count in percentages, MCH (pg)- mean corpuscular hemoglobin in picogram.

Biochemical Parameters

Random Blood Sugar (RBS), Testosterone, and Cortisol were examined in the diagnostic laboratory.

Statistical Analysis

The data were analyzed using the statistical software program SPSS 20.0 for Windows 7. One-way ANOVA was performed to assess the significant difference among different groups in terms of physiological and hematological parameters. Data were expressed as mean ± standard deviation (SD). $P<0.05$ was considered statistically significant.

RESULTS

Two groups of rabbits (n=10) were injected with two different anesthetic protocols following an experimental design. In group XK, 30 minutes after administration of the xylazine and ketamine hydrochloride combination during neutering, no use of atipamezole hydrochloride was made, and in group XKA, atipamezole was used after 30 minutes of xylazine-ketamine anesthesia to find the effects of atipamezole on physiological and hematological parameters of the rabbits during neutering.

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Clinical Parameters

The effects of anesthetic agents on physiological parameters in rabbits were presented in Tables 1 to 3.

Effects of Anesthesia on Heart Rate in Rabbits

Table-1 shows that the mean values of heart rate per minute before anesthesia, 15 min, 30 min, and 45 min post-injection in the XK group were 230±15.81, 193±14.83, 166±24.08, and 195.2±12.5 min, respectively, indicating that the heart rate decreased gradually up to 30 min of anesthesia and almost regained a normal level at 45 min post-anesthesia. The mean values of heart rate per minute before anesthesia, at 15 min, 30 min, and 45 min after post-injection in the XKA group were 246±35.78, 190±15.81, 162±22.80, and 200±15.81, respectively. It also indicates almost the same changes in group XK, except that at 45 min the heart rate sharply regained normalcy.

Table 1. Effects of anesthetic agents on heart rate (per min) in rabbits

Group	Before anesthesia	15 min post-injection	30 min post-injection	45 min post-injection
XK	230±15.81	190±15.81**	162±22.80**	200±15.81*
XKA	246±35.78	193±14.83**	166±24.08**	195.2±12.5**

Effects of anesthesia on respiration rate in Rabbits

Table-2 shows that the mean values of respiration rate per minute before anesthesia, 15 min, 30 min, and 45 min post-injection in the XK group were 89.8±3.96, 53±10.9, 56±5.09, and 93.2±2.39, respectively, indicating that the respiration rate decreased gradually up to 15 min of anesthesia and gradually increased from 30 min to 45 min post-anesthesia. The mean values of respiration rate per minute before anesthesia, at 15 min, 30 min, and 45 min after post-injection in the XKA group were 89±6.04, 64.6±2.2, 66±8.37, and 91±3.39 min, respectively. It also indicates almost the same changes in group XK.

Table 2. Effects of anesthetic agents on respiration rate (per min) in rabbits

Group	Before anesthesia	15 min post-injection	30 min post-injection	45 min post-injection
XK	89.8±3.96	53±10.9**	56±5.09**	93.2±2.39
XKA	89±6.04	64.6±2.2**	66±8.37**	91±3.39

Effects of anesthesia on rectal temperature in Rabbits

Table-3 shows that the mean values of rectal temperature (ºF) before anesthesia, 15 min, 30 min, and 45 min post-injection in the XK group were 101.9±0.63, 100.3±0.93, 100.54±0.46, and 101.1±0.74, respectively, indicating that the rectal temperature (ºF) decreased gradually up to 30 min of anesthesia and almost regained a normal level at 45 min post-anesthesia. The mean values of the rectal temperature (ºF) before anesthesia, at 15 min, 30 min, and 45 min after post-injection in the XKA group were102.2±0.91, 99.5±1.94, 99.2±2.17, 101±0.61°F, respectively. It also indicates almost the same changes in group XK.

Table 3. Effects of anesthetic agents on the rectal temperature (ºF) in rabbits

Group	Before anesthesia	15 min post-injection	30 min post-injection	45 min post-injection
XK	101.9±0.63	100.3±0.93	100.54±0.46**	101.1±0.74
XKA	102.2±0.91	99.5±1.94*	99.2±2.17**	101±0.61

For heart rate (Table 1), there were no statistically significant differences between the XK and XKA groups at any time point (pre-anesthesia: $p = 0.42$; 15 min: $p = 0.76$; 30 min: $p = 0.78$; 45 min: $p = 0.67$; independent t-tests). For respiration rate (Table 2), the XKA group had a significantly higher rate at 15 min (64.6±2.2 vs. 53±10.9 breaths/min; $p = 0.04$) and at 30 min (66±8.37 vs. 56±5.09 breaths/min; $p = 0.03$), but not at other

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time points. For rectal temperature (Table 3), there were no statistically significant differences between the groups at any time point ($p > 0.05$ for all comparisons). These results indicate that while atipamezole improved recovery time, its effects on heart rate and temperature were limited, consistent with its primary mechanism as an alpha-2 antagonist rather than a direct cardiostimulant.

Induction period, duration of anesthesia, and recovery period from Anesthesia

Anesthetic agents were applied according to the body weight of the animals. Effects of anesthetic agents on the induction period, duration of anesthesia, and recovery from anesthesia without atipamezole and with atipamezole were recorded in rabbits. Table 4 shows that the induction period of anesthesia was 12.4±1.52 min and 13.2±2.17 min in group XK and XKA, respectively. In group XKA, the duration of anesthesia was shorter (45.7±0.84 min) than that in group XK (59.5±4.27 min), indicating that atipamezole reduced the duration of anesthesia. The recovery period was significantly longer in the XK group (153.0±4.47 min) compared to the XKA group (86.0±9.6 min; $p = 0.00006$), as shown in Table 4, indicating that atipamezole reduced the recovery period.

Table 4. Anesthetic doses, induction period, duration of anesthesia, and Recovery period

Group	Induction period (min)	Duration of anesthesia (min)	Recovery period (min)
XK	12.4±1.52	59.5±4.27	153±4.47
XKA	13.2±2.17	45.7±0.84**	86±9.6**
P-Value	0.518	0.0001	0.00006

Effects of anesthesia on hematological parameters in rabbits

Figure 2 shows that, before anesthesia, the Hb levels were 9.92±0.896 and 10.3±0.55 in the XK and XKA groups, respectively. After 45 minutes, the Hb levels were decreased to 9.22±0.66 ($P \leq 0.05$) and 9.5±0.47 in both groups. The Hb levels were increased after recovery to 9.68±0.82 and 9.82±0.40 in XK and XKA groups, respectively.

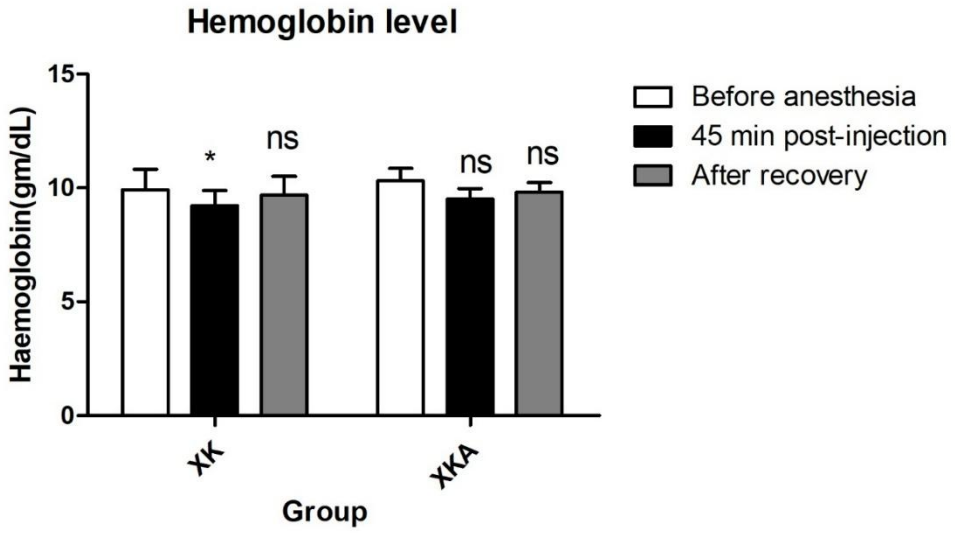

Figure 2. Comparison of hemoglobin levels(gm/dL) between group XK and XKA before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

Figure 3 shows that, before anesthesia, the RBC levels were 5.45±0.11 and 5.37±0.105 in group-XK and XKA, respectively. After 45 minutes of post-injection, the levels of RBC were 5.434±0.68 and 5.49±0.40 in group-

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XK and XKA, respectively. After recovery, RBC levels were 5.02±0.35 and 5.61±0.31 in group-XK and XKA, respectively. No significant changes were in the RBC level.

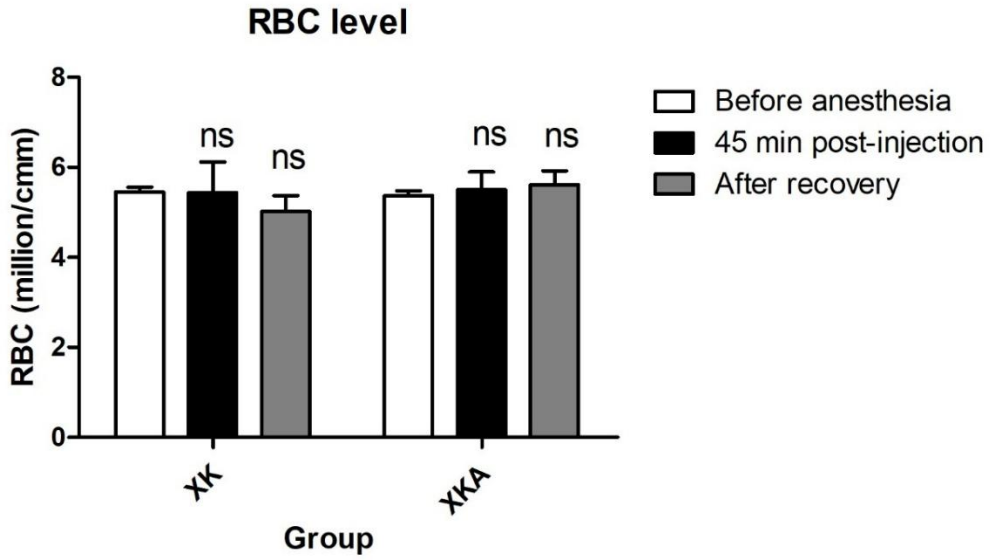

Figure 3. Comparison of RBC (million/cmm) between group XK and XKA before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

Figure 4 shows that, before anesthesia, the WBC levels were 11300±547and 11500±547 in group-XK and XKA, respectively. After 45 minutes, the levels of WBC were decreased in 8660±1952.7 ($P \leq 0.01$) and 8260±2184.7 ($P \leq 0.01$) in both group A and B. The WBC levels were also decreased after recovery in 8482±211 **(**$P \leq 0.01$) and 8359±456 ($P \leq 0.01$) in group-XK and XKA, respectively.

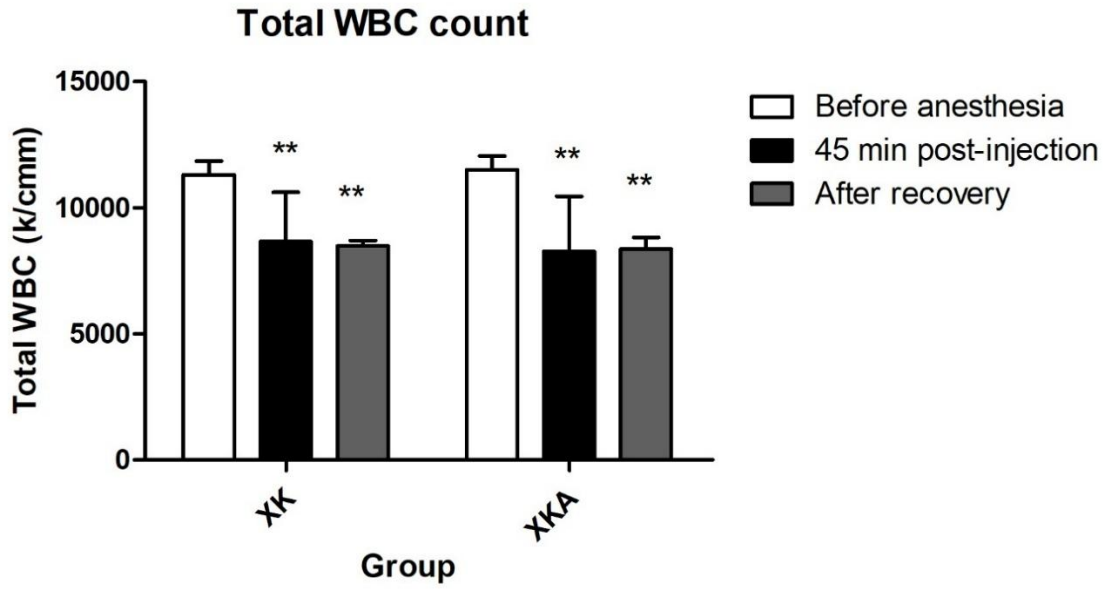

Figure 4. Comparison of Total WBC Count(k/cmm) between group XK and XKA before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

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Figure 5 shows that, before anesthesia, the neutrophil levels were 28.5±0.58and 28.25±0.96 in group-XK and XKA, respectively. After 45 minutes, the levels of neutrophil were 30.4±1.14 and 30±1.58, and after recovery, it was 30±3.8 and 30±2.23 in group-XK and XKA, respectively. No significant changes were in the neutrophil level.

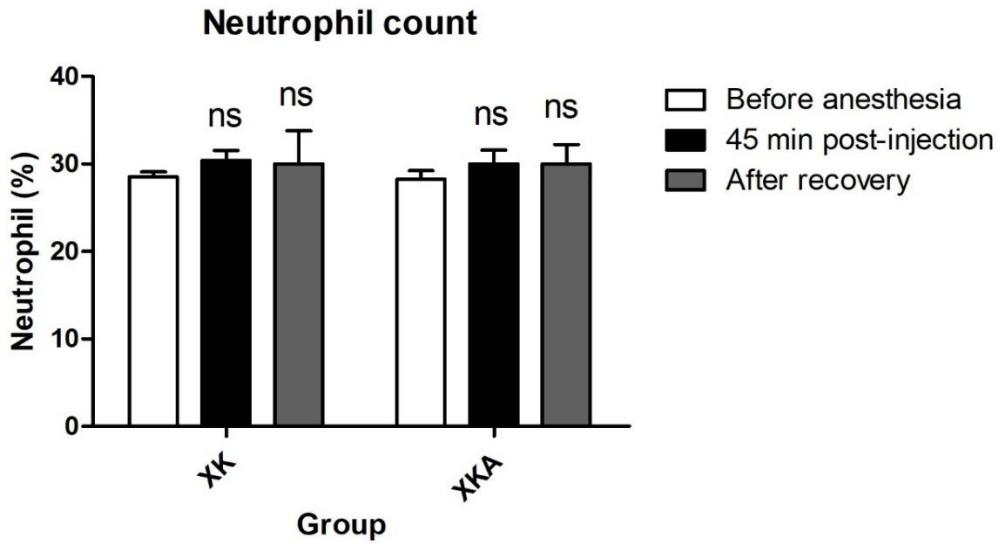

Figure 5. Comparison of Neutrophils (%) between XK and XKA groups before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

Figure 6 shows that, before anesthesia, the lymphocyte levels were 79.6±1.34 and 80.01±1.0 in group-XK and XKA, respectively. After 45 minutes, the levels of lymphocytes were decreased to 50.4±5.59 ($P \leq 0.01$) and 65.2±15.77 ($P \leq 0.01$) in both groups. After recovery, it was 59.6±9.39 ($P \leq 0.05$) and 48.6±6.54 ($P \leq 0.01$) in group-XK and XKA, respectively.

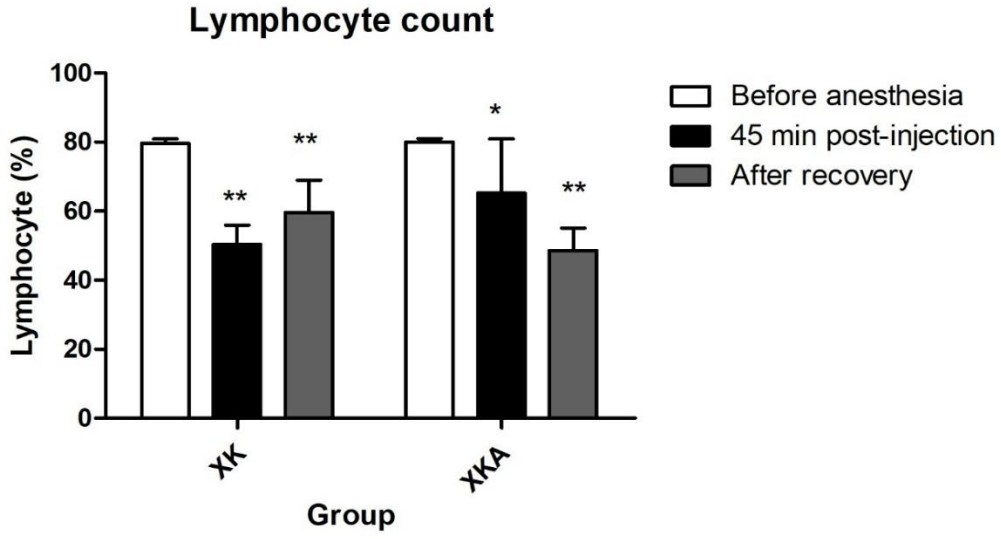

Figure 6. Comparison of Lymphocytes (%) between XK and XKA groups before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

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Figure 7 shows that, before anesthesia, the monocyte levels were 16.4±0.55 and 16.6±0.55 in group-XK and XKA, respectively. After 45 minutes, the monocyte levels were decreased to 15.8±0.84 and 15.8±1.09. The monocyte levels were increased after recovery of neutering at 16.2±2.58 and 16.4±2.5.

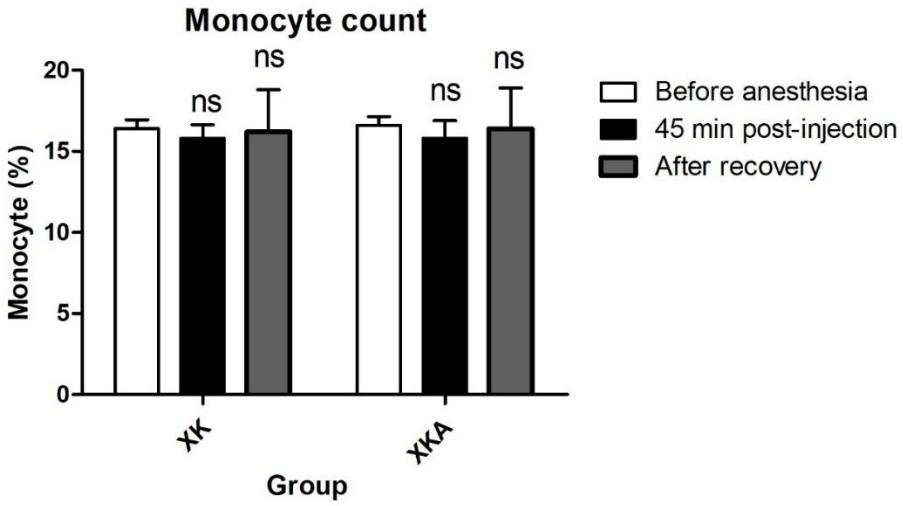

Figure 7. Comparison of Monocytes (%) between XK and XKA groups before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.

Before anesthesia, the eosinophil levels were 3.0±0.00 and 3.0±0.00 in group-XK and XKA, respectively. After 45 minutes, the eosinophil level in the XK group was 3.0±0.83, and in group XKA increased to 4.2±0.84 ($P \leq 0.05$). The eosinophil levels were significantly increased after recovery of neutering at 3.8±0.83 and 4.4±1.14 ($P \leq 0.05$) in group-XK and XKA, respectively (Supplementary figure 5).

Table-8 shows that, before anesthesia, the PCV levels were 49.6.5±7.3 and 50.4±4.33 in group-XK and XKA, respectively. After 45 minutes, the PCV levels were increased at 55.2±1.3 and 52.0±5.24 in group-XK and XKA, respectively. The PCV levels were increased after recovery from anesthesia to 58±3.16 ($P \leq 0.05$) and 55±3.53 in group-XK and XKA, respectively.

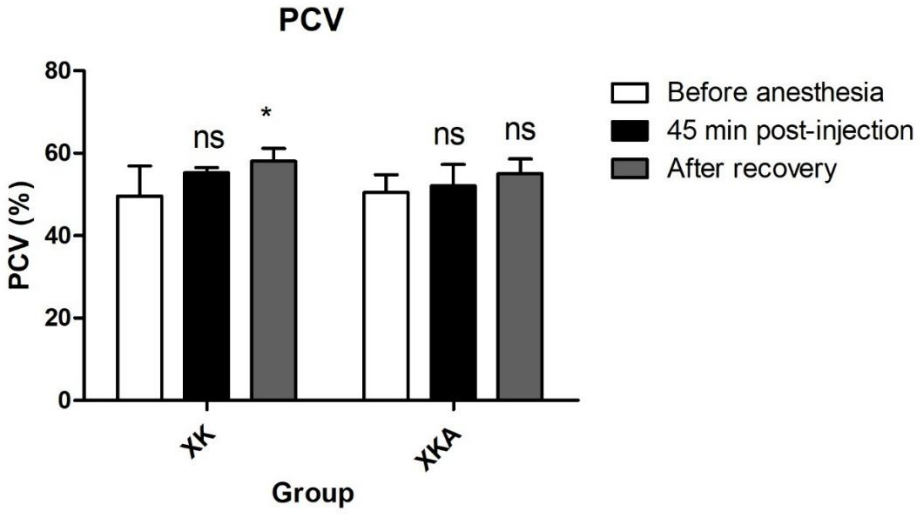

Figure 8. Comparison of PCV (%) between XK and XKA groups before and after induction of anesthesia. The data from two groups were analyzed using the compare means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via the one-way ANOVA test. All of the results are expressed as the mean ± SD. *

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indicates P ≤ 0.05 and ** indicates P ≤ 0.01. A P-value ≤ 0.05 between the two groups was considered significant.

Table 5 shows that, before anesthesia, the RBS levels were 2.01±0.04 and 2.52±0.48 in group-XK and XKA, respectively. After 45 minutes, the RBS levels were decreased at 1.72±0.13 (P ≤ 0.01) and 1.65±0.10 (P ≤ 0.01), respectively. The RBS levels were 1.82±0.13 (P ≤ 0.05) and 1.76±0.27 (P ≤ 0.01) in group-XK and XKA, respectively, after recovery.

Table 5. Comparison of RBS (mmol/L) before and after induction of anesthesia

Group	Before anesthesia	45min post-injection	After recovery
XK	2.01±0 .04	1.72± 0.13**	1.82±0.13*
XKA	2.52±0. .48	1.65± 0.10**	1.76±0.27**

Table 6 shows that, before anesthesia, the cortisol levels were 3.98±0.19 and 3.89±0.16 in the control and treatment groups, respectively. After 45 minutes, the levels of cortisol were increased at 5.072±0.07 (P ≤ 0.01) and 5.79±0.35 (P ≤ 0.01) in group XK and XKA, respectively. After recovery, the levels of cortisol were 5.9±0.31(P ≤ 0.01) and 6.0±0.49 (P ≤ 0.01) in group XK and XKA, respectively.

Table 6. Comparison of Cortisol(µg/dl) before and after induction of anesthesia

Group	Before Anesthesia	45min post-injection	After Recovery
XK	3.93±0.18	5.07±0.07**	5.90±0.31**
XKA	4.01±0.19	5.79±0.35**	6.0±0.49**

Effects of anesthesia on male sex hormones in rabbits

Table 7 shows that, during the normal condition of rabbits, the testosterone levels were 0.38±0.06 and 0.32±0.06 in group-XK and XKA, respectively. After 45 minutes, the levels of testosterone were increased at 1.74±0.85 (P ≤ 0.01) and 0.56±0.10 (P ≤ 0.01) in the control and target group, respectively. After recovery, the levels of testosterone were 0.70±0.56 and 0.6±0.08 (P ≤ 0.01) in the respective groups.

Table 7. Comparison Testosterone (pg/ml) before and after induction of anesthesia

Group	Before Anesthesia	45min post-injection	After Recovery
XK	0.38±0.06	1.74±0.85**	0.70±0.56ns
XKA	0.32±0.06	0.56±0.10**	0.60±0.08**

Reflex monitored during the experiments

The depth of anesthesia was monitored by using of various reflexes. The palpebral reflex, corneal reflex, and pedal reflexes were present in both groups at 05 min of anesthesia. In deep anesthetic periods at 15 min, the pedal reflex becomes sluggish and almost absent within the period of anesthesia extended up to 20 to 25 min. The reflexes became normal at the recovery stage.

Observation of defecation, urination, salivation, and lacrimation during the experiments

In this study, there was no salivation and lacrimation in both groups. Defecation was observed in group XKA, and urination was observed in group XK.

Observation of healing

On the second day, heat and swelling were observed at the site in the control group. On that day, a few swellings were observed in the rabbits of the target group. On the third day, there was no heat at the site, and

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the swelling had decreased compared to the previous day in both groups. On the seventh day, healing was good in both groups (Supplementary figure 4).

Complications

No complication occurred, such as hemorrhage, infection, dehiscence, adhesions, stress-anxiety, etc.

DISCUSSION

The present study was conducted to evaluate the effect of atipamezole on recovery of the rabbits from xylazine–ketamine induced anesthesia during neutering in male rabbits, with particular emphasis on recovery characteristics, physiological responses, and blood parameters. Xylazine–ketamine is a commonly used anesthetic combination in rabbits; however, its use is often associated with prolonged recovery, cardiovascular depression and metabolic disturbances (Flecknell, 2020). The findings of the present study indicate that atipamezole significantly improves recovery quality and facilitates normalization of altered physiological and hematological parameters.

In the present study, rabbits treated with atipamezole showed significantly shorter recovery times compared to those recovering without a reversal agent. Time to regain righting reflex, sternal recumbency, and standing was markedly reduced following atipamezole administration. Similar findings have been reported in rabbits and other laboratory animals, where atipamezole effectively reversed α_2-agonist-induced sedation and hastened recovery (Kim et al., 2004).

Xylazine produces sedation and muscle relaxation through stimulation of central α_2-adrenergic receptors, leading to suppression of sympathetic nervous activity (Giovannitti et al., 2015; Sinclair, 2003).

Atipamezole, a selective α_2-adrenergic antagonist, competitively blocks these receptors, thereby reversing the sedative and cardiovascular effects of xylazine (Virtanen et al., 1988). Although ketamine is not directly antagonized, reversal of the xylazine component is sufficient to restore consciousness and mobility, as previously described by Hedenqvist et al., 2013. Rapid recovery is particularly advantageous in rabbits, which are highly sensitive to prolonged anesthesia and post-anesthetic stress. Delayed recovery may predispose rabbits to hypothermia, gastrointestinal stasis and respiratory compromise (Oglesbee & Lord, 2020).

Xylazine–ketamine anesthesia in the present study caused a decrease in heart rate, respiratory rate and rectal temperature during the anesthetic period. These findings are consistent with earlier studies reporting bradycardia, respiratory depression and hypothermia following xylazine administration in rabbits (Gadelrab et al., 2025). Following administration of atipamezole, physiological parameters improved significantly and gradually returned toward baseline values. The increase in heart rate and respiratory rate observed during recovery can be attributed to the restoration of sympathetic tone after α_2-receptor blockade (Madirazza et al., 2022). Similar improvements in cardiopulmonary function following atipamezole administration have been documented in rabbits and other species (Karin et al., 2006).

Normalization of rectal temperature after reversal may be associated with increased muscular activity and metabolic rate during recovery. Hypothermia is a common complication of rabbit anesthesia, and faster recovery contributes to reduced heat loss and improved thermoregulation (Flecknell, 2020).

The present study revealed differences in hematological parameters before and after administration of xylazine–ketamine anesthesia. In case of group XKA, a reduction in hemoglobin concentration, WBC, platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils, packed cell volume (PCV), RBC, MCV, MCH, MCHC, RDW, and RBS was observed before and after anesthesia.

Similar reductions have been attributed to splenic sequestration of erythrocytes and hemodilution caused by peripheral vasodilation and altered vascular dynamics during anesthesia (Grover & Loegering, 1982; Green, 1981).

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Total leukocyte count and differential leukocyte values exhibited mild fluctuations, likely due to stress-induced endocrine responses. Surgical stress and anesthesia stimulate the release of corticosteroids and catecholamines, which influence leukocyte distribution, often resulting in neutrophilia and lymphopenia (Walther et al., 2024; Burdine, 2002).

In the case of group XK, Differences were also observed before and after application of the anesthetic agents. A reduction in hemoglobin concentration, WBC, platelets, lymphocytes, monocytes, eosinophils, basophils, packed cell volume (PCV), RBC, MCV, MCH, MCHC, RDW, and RBS was observed before and after anesthesia. However, neutrophils, monocytes, eosinophils, basophils, packed cell volume (PCV), testosterone, and cortisol levels increased after administration of the anesthetic agents.

Following administration of atipamezole, hematological parameters gradually returned toward pre-anesthetic values. Restoration of normal vascular tone and splenic contraction following α_2-antagonism may have facilitated redistribution of erythrocytes into the peripheral circulation (Talke et al., 2000; Vähä-Vahe, 1990). These findings agree with previous reports demonstrating improved hematological stability following reversal of α_2-agonist effects (Ali, 2013).

Xylazine–ketamine anesthesia resulted in a transient increase in blood glucose levels in the present study. Testosterone and cortisol levels were also increased after administration of the anesthetic agents.

This hyperglycemic effect is well documented and is primarily due to xylazine-induced inhibition of insulin secretion and increased hepatic glucose production (Khokhlova et al., 2022; Mohammed et al.,2012). Mild elevations in serum ALT and AST observed after anesthesia may reflect temporary hepatic stress or reduced hepatic perfusion rather than direct hepatocellular damage. Similar transient increases in liver enzyme activities following anesthesia have been reported in rabbits and other laboratory animals (Afaf et al., 2024; Oh et al., 2020). Minor fluctuations in serum urea and creatinine levels may be related to altered renal blood flow during anesthesia (Green, 1981; Saran et al., 2025). Anesthesia and surgical stress are known to influence endocrine function, including testosterone secretion. Injectable anesthetic agents such as xylazine–ketamine activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in increased secretion of stress hormones, particularly cortisol. Elevated cortisol levels can suppress the hypothalamic–pituitary–gonadal (HPG) axis, leading to alterations in luteinizing hormone (LH) release and subsequent changes in circulating testosterone concentrations.

Several studies have reported transient fluctuations in testosterone levels following anesthesia, which may include a short-term increase due to an acute stress response, followed by a decline during the recovery phase (Meyer, 2017). These changes are generally temporary and depend on the type of anesthetic agent, duration of anesthesia, and intensity of surgical stress.

Neutering (orchiectomy) results in a marked and permanent reduction in testosterone levels due to the removal of the testes, the primary source of androgen production. Following neutering, serum testosterone levels decline rapidly, leading to suppression of sexual behavior, reduced aggression, and decreased urine spraying in male rabbits (Nishiyama, 2014).

The reduction in testosterone after neutering is considered beneficial for animal welfare and is one of the primary physiological outcomes evaluated in studies involving male rabbit castration (Rayner et al., 2025).

Anesthesia, particularly injectable anesthetic protocols involving α_2-adrenergic agonists such as xylazine or medetomidine, induces activation of the hypothalamic–pituitary–adrenal (HPA) axis. Surgical stress and tissue manipulation during procedures like neutering further stimulate the release of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), resulting in elevated circulating cortisol levels (Ivascu et al., 2024; Cusack & Buggy, 2020).

Several studies have demonstrated a significant increase in serum cortisol levels following xylazine–ketamine anesthesia, which is attributed to the combined effects of anesthetic stress, surgical trauma, and altered neuroendocrine regulation (Ali, 2013).

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Elevated cortisol levels during and after anesthesia reflect the intensity of stress experienced by the animal.

Neutering is a surgical intervention that induces acute stress, leading to a transient rise in cortisol levels in male rabbits. Increased cortisol concentrations observed immediately after neutering indicate activation of the stress response system. However, these elevations are generally short-lived and decline as recovery progresses, and pain is adequately managed (Pieper et al., 2025).

Surgical procedures such as neutering activate the hypothalamic–pituitary–adrenal (HPA) axis, leading to increased secretion of cortisol and catecholamines. These stress hormones promote gluconeogenesis and reduce peripheral glucose uptake, resulting in elevated RBS levels during the perioperative period (Rayner et al., 2025).

Administration of atipamezole facilitated faster normalization of biochemical parameters, including blood glucose and liver enzyme levels. Restoration of sympathetic activity improves tissue perfusion and metabolic balance, contributing to recovery of normal biochemical profiles (Flecknell, 2020).

The findings of the present study highlight the clinical benefits of using atipamezole as a reversal agent in xylazine–ketamine anesthesia protocols for rabbits. Faster recovery, improved physiological stability, and normalization of blood parameters reduce post-anesthetic complications and enhance animal welfare. These advantages are particularly important during routine surgical procedures such as neutering, where rapid and smooth recovery is desirable (Ali, 2013). Proper timing and dosage of atipamezole are essential to avoid premature arousal during surgery. When administered after completion of the procedure, atipamezole appears to be safe and effective in rabbits (Green, 1981).

CONCLUSION

The present study demonstrates that the xylazine–ketamine anesthetic protocol, followed by reversal with atipamezole, provides a safe, effective, and reversible anesthetic regimen for rabbits undergoing neutering surgery. Xylazine–ketamine produced adequate surgical anesthesia with predictable physiological alterations, while atipamezole effectively facilitated recovery by antagonizing alpha-2 adrenergic effects.

Hematological changes observed during anesthesia were transient and primarily associated with stress-related leukocyte redistribution and mild erythrocyte alterations, with no evidence of clinically significant compromise. The partial normalization of leukocyte profiles following atipamezole administration confirms its efficacy in reversing xylazine-induced immunomodulatory effects. Red blood cell indices showed minimal immediate response to reversal, indicating limited short-term influence of alpha-2 antagonism on erythrocyte parameters.

Biochemical parameters, including random blood glucose, remained within normal physiological limits throughout the study, reflecting metabolic stability during anesthesia and recovery. Endocrine evaluation revealed suppression of cortisol and testosterone concentrations during anesthesia, followed by partial restoration after atipamezole administration, highlighting the reversible neuroendocrine effects of alpha-2 agonists.

Overall, the findings support the use of xylazine–ketamine anesthesia with atipamezole reversal as a reliable protocol for rabbit neutering, offering adequate surgical depth, smooth recovery, and minimal adverse physiological effects. This protocol is particularly advantageous in both experimental and clinical settings where rapid recovery and improved animal welfare are priorities.

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AUTHOR CONTRIBUTIONS

ACR and SA designed outlines and drafted the manuscript. SA, MSR, SHL, CRD, and AH performed the experiments, and ACR and SA analyzed the data. SA and ACR wrote the initial draft of the manuscript. ACR, BP, SR, and SA reviewed the scientific contents described in the manuscript. All authors read and approved the final submitted version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

SUPPLEMENTARY MATERIALS

Supplementary Figure 1. The drugs used in the experiment, Supplementary Figure 2. Preoperative preparation of the rabbits; Supplementary figure 3. Recording the clinical parameters and the procedure of blood collection; Supplementary figure 4. Healing condition day by day and Supplementary Figure 5. Comparison of Eosinophils (%) between XK and XKA groups before and after induction of anesthesia.

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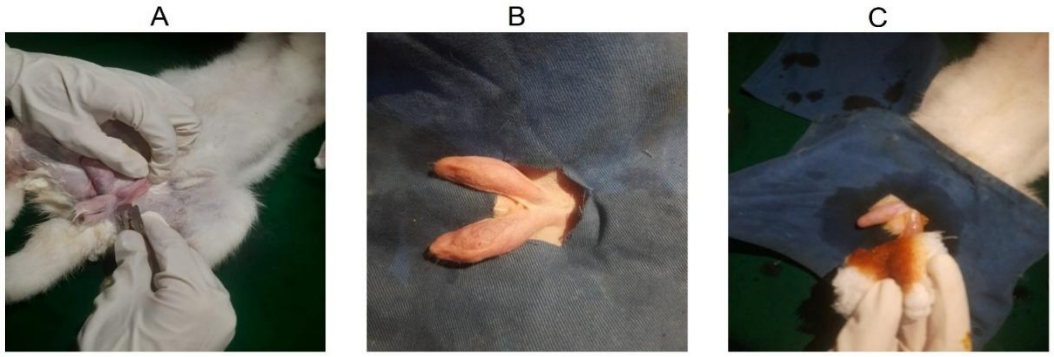
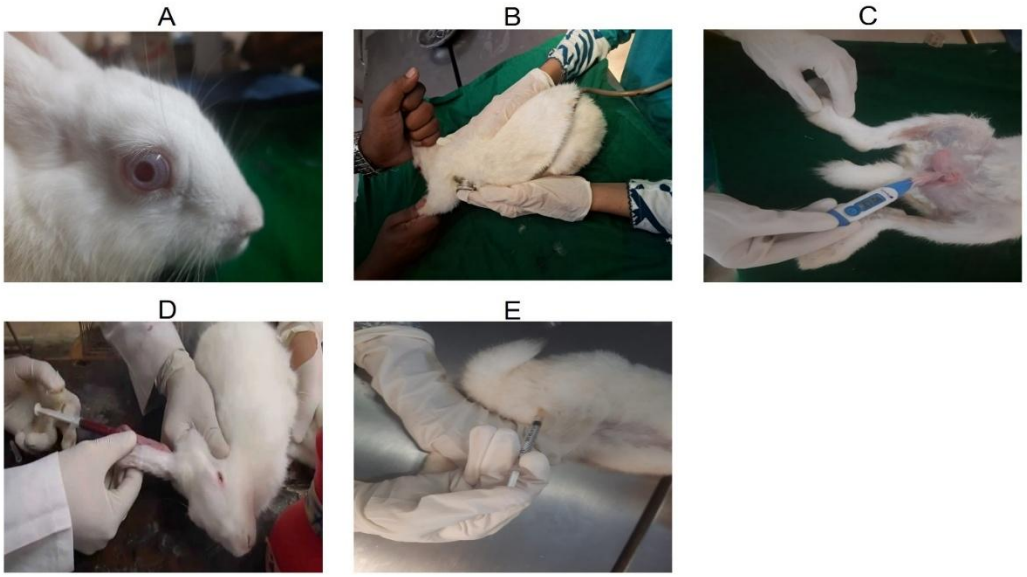
APPENDIX

Supplementary Figure 1. The drugs used in the experiment. Xylazine HCl and ketamine HCl were used for anesthesia of rabbits, and atipamezole was used to observe the effect on the recovery of the rabbits.



Supplementary Figure 2. Preoperative preparation of the rabbits. A) Shaving the site of operation, B) Draping the rabbits, and C) site preparation with povidone iodine.



Supplementary Figure 3. Recording the clinical parameters and the procedure of blood collection. A) a healthy male rabbit, B) Recording of heart rate by using a stethoscope, C) Recording the rectal temperature using the digital thermometer, D) Collection of blood from the ear vein, and E) Injection of an anesthetic agent intramuscularly.

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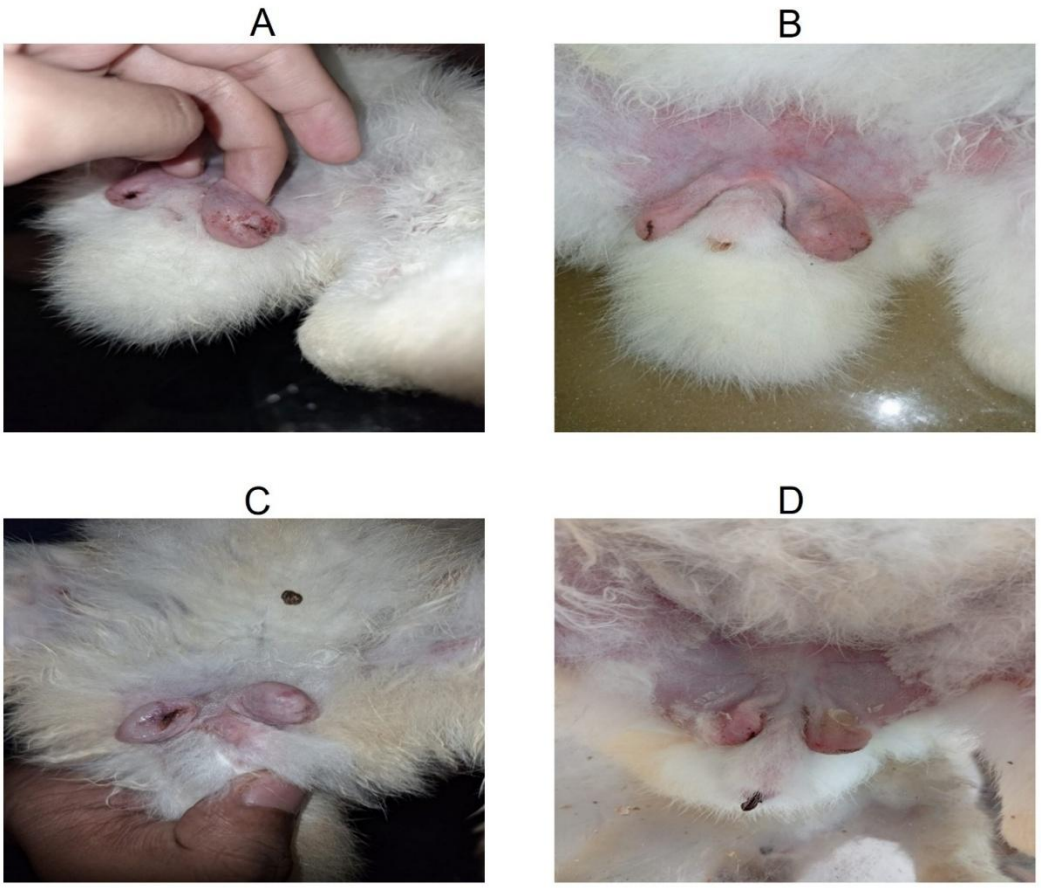
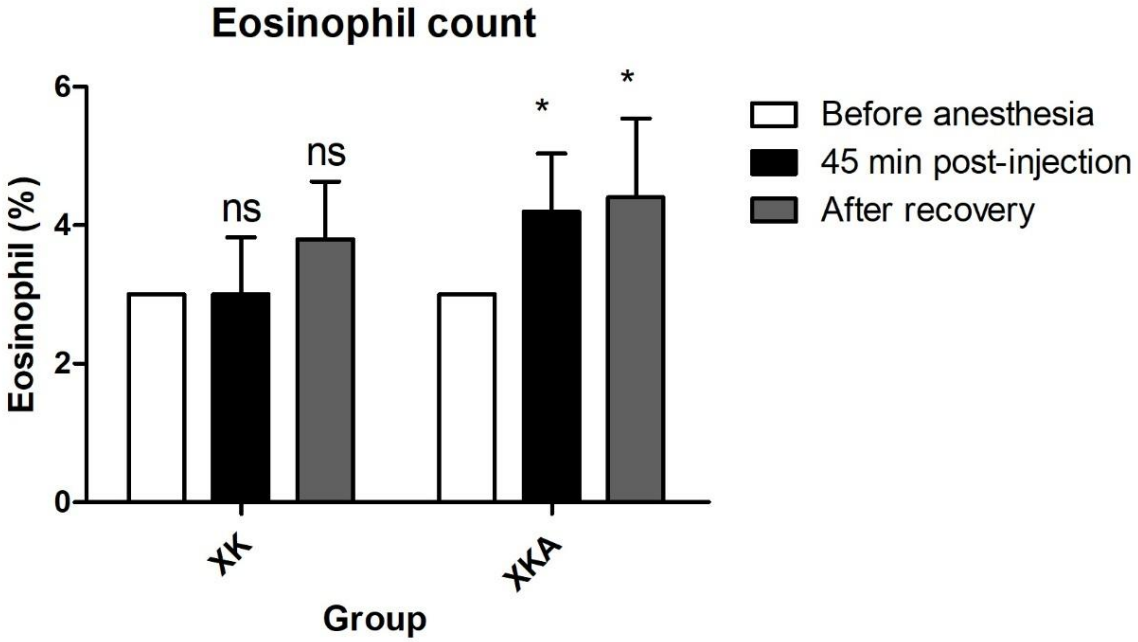


Supplementary figure 4. Healing condition day by day. A) Healing status on 1st day, B) Healing status on 3rd day, C) Healing status on 5th day, and D) Healing status on 7th day.



Supplementary figure 5. Comparison of Eosinophils (%) between XK and XKA groups before and after induction of anesthesia. The data from two groups were analyzed using the Compare Means feature in IBM SPSS Statistics for Windows (IBM Corp., NY, USA) via a one-way ANOVA. All of the results are expressed as the mean ± SD. * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$. A P-value ≤ 0.05 between the two groups was considered significant.