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Comparison of clinical effects of four different anesthesia protocols in rabbits

Comparación de los efectos clínicos de cuatro protocolos de anestesia diferentes en conejos

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ABSTRACT

The aim of this study is to compare the clinical effects of four different anesthesia protocols in rabbits and to suggest a safe and controlled alternative inhalation anesthesia with endotracheal intubation technique for clinical applications and experimental studies in rabbits. A total of 40 New Zealand rabbits were randomly divided into four groups (n = 10). Propofol-sevoflurane, midazolam-sevoflurane, medetomidine-ketamine-sevoflurane and sevoflurane only. All anesthesia protocols were completed without causing death. Although the medetomidine-ketamine-sevoflurane provided the best surgical anesthesia (2.75 ± 0.5) and induction quality (2.90 ± 0.5), it caused significant decreases in HR and RR from baseline (230.6 ± 9.9 and 89.5±7.9, respectively) to the 10th min of anesthesia (167.1 ± 12.7 and 45.8 ± 4.9, respectively) ($P < 0.05$). The fastest anesthesia induction was observed in the propofol-sevoflurane (2.5 ± 0.5), while the medetomidine-ketamine-sevoflurane had the longest recovery time (84,0 ± 8,1). In the midazolam-sevoflurane group, intubation was significantly more difficult ($P < 0.05$). No significant difference was found between the groups in terms of post-anesthesia hematological effects. Based on the superior analgesic depth observed in this study and supported by previous literature, the medetomidine-ketamine-sevoflurane combination may be considered a suitable option for rabbits undergoing complex procedures. However, continuous monitoring of physiological parameters during and after anesthesia until full recovery is essential to prevent potential complications. Additionally, the propofol-sevoflurane combination appears to be a safe and suitable option for rabbits requiring imaging or immobilization, as it allows for rapid recovery and awakening.

Key words: Anesthesia; intubation; rabbit; recovery; sevoflurane.

RESUMEN

El objetivo de este estudio es comparar los efectos clínicos de cuatro protocolos de anestesia diferentes en conejos y sugerir una alternativa segura y controlada de anestesia inhalatoria con intubación endotraqueal para aplicaciones clínicas y estudios experimentales en conejos. Un total de 40 conejos de Nueva Zelanda se dividieron aleatoriamente en cuatro grupos (n = 10): propofol-sevoflurano, midazolam-sevoflurano, medetomidina-ketamina-sevoflurano y sevoflurano solo. Todos los protocolos de anestesia se completaron sin causar la muerte. Aunque el medetomidina-ketamina-sevoflurano proporcionó la mejor anestesia quirúrgica (2.75 ± 0.5) y calidad de inducción (2.90 ± 0.5), causó disminuciones significativas en la frecuencia cardíaca y la frecuencia respiratoria desde el inicio (230.6 ± 9.9 y 89,5 ± 7,9, respectivamente) hasta el décimo minuto de anestesia (167.1 ± 12.7 y 45.8 ± 4.9, respectivamente) ($P < 0.05$). La inducción de anestesia más rápida se observó en el propofol-sevoflurano (2.5 ± 0.5), mientras que el medetomidina-ketamina-sevoflurano tuvo el tiempo de recuperación más largo (84.0 ± 8.1). En el grupo midazolam-sevoflurano, la intubación fue significativamente más difícil ($P < 0.05$). No se encontraron diferencias significativas entre los grupos en términos de efectos hematológicos postanestésicos. Basándose en la mayor profundidad analgésica observada en este estudio y respaldada por la literatura previa, la combinación medetomidina-ketamina-sevoflurano puede considerarse una opción adecuada para conejos sometidos a procedimientos complejos. Sin embargo, la monitorización continua de los parámetros fisiológicos durante y después de la anestesia hasta la recuperación completa es esencial para prevenir posibles complicaciones. Además, la combinación propofol-sevoflurano parece ser una opción segura y adecuada para conejos que requieren imágenes o inmovilización, ya que permite una rápida recuperación y despertar.

Palabras clave: Anestesia; intubación; conejo; recuperación; sevoflurano.

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INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are widely accepted as suitable animal models for experimental and biomedical research due to their phylogenetic similarity to humans. They are particularly valuable in pharmacological, toxicological, surgical, and genetic studies, owing to several advantages such as their docile nature, ease of handling, low maintenance requirements, economic value, and the presence of large ear veins [1, 2].

In rabbits, as in other animals, injectable and inhalation anesthesia methods are used in anesthesia. Inhalation anesthesia agents are widely used as the sole source of anesthesia in small guinea pigs (*Cavia porcellus*), while injectable agents are often used in combination with inhalation anesthesia for rabbits and larger guinea pigs (*Cavia magna*). Short-term general anesthesia in rabbits can be safely applied with injectable anesthetic agents, anesthetic combinations of these agents with sedative, tranquilizer and analgesics. However, experience gained by researchers has shown that the analgesic properties of these combinations are ineffective in major operations and in many cases cause significant hypotension, which can increase mortality. Furthermore, rabbits show very wide individual (both racial and gender-specific) differences in response to anesthetics [3, 4].

The effects of inhalation agents are easily reversible, the depth of anesthesia can be adjusted, and the experimental results are minimally affected. Inhalation anesthesia can be applied via mask induction following chamber or injectable anesthetic induction and can be maintained through endotracheal intubation techniques [5, 6].

The aim of this presented study is to compare the clinical effects of the anesthesia with propofol-sevoflurane (P-S), midazolam-sevoflurane (Mi-S) and medetomidine-ketamine-sevoflurane(Me-K-S) and sevoflurane-only (S) protocols in rabbits and to suggest a safe and controlled alternative inhalation anesthesia with endotracheal intubation technique for clinical applications and experimental studies in rabbits.

MATERIALS AND METHODS

This study was conducted with the approval of the Erciyes University Animal Experiments Local Ethics Committee (EUHADYEK, Approval No: 17/016). A total of 40 New Zealand rabbits of varying weights (BW 3-4.5 kg) and ages (2–3 years) were included. The rabbits were randomly divided into four equal groups of 10 animals each. Animals were weighed on the day (d) of the experiment and healthy animals were included in the study. Vascular access was provided through the marginal ear vein in all animals.

In the study, midazolam (Demizolam, Delta Select GmbH, Germany), medetomidine (Domitor, Pfizer, Germany), ketamine (Alfamine, Alfasan, Nederland), propofol (Propofol 1 % Fresenius, Fresenius Kabi, Germany) and sevoflurane (Sevoflurane, AbbVie, Italy) were applied.

In the P-S group; propofol 7 mg/kg BW IV was administered through the marginal ear vein, in the Mi-S group; midazolam 0.3 mg/kg BW IM, in the Me-K-S group; medetomidine 0.3 mg/kg bw IM and then ketamine 30 mg/kg bw IM 3 minutes (min) later. In these three groups, rabbits were intubated after the injections and sevoflurane was administered as 4 % with 500 ml/kg/min oxygen for 30 min. In the S group, only sevoflurane was administered by mask as 4 % with 500 ml/kg/min oxygen for 30 min.

After the use of injectable induction agents, intubation was performed with a size 2.5 cuffed endotracheal tube (Hitec Medical, China). Inhalant anesthetic agent sevoflurane was applied for 30 min using an anesthesia device (Maxi 2200, TMS, Türkiye) and a non-rebreathing circuit (Magill type) system. After this 30-min application, the rabbits were separated from the anesthesia device.

No surgical intervention was performed in the study. Anesthesia was evaluated by response to pain and reflex tests, intubation tolerance, cardiopulmonary parameters and BT.

In all animals, physiological parameters were measured 30 min before starting anesthesia (Control, t = -30). Sampling times were performed every 10 min (t = 10, 20, 30) during the 30-min sevoflurane continuation following the injections (t = 0) and at 15, 30, 60, 90 min after the end of sevoflurane (t = 45, 60, 90, 120, respectively) and the animals were monitored. Heart rate (HR) and peripheral capillary oxygen saturation (SpO2) were measured using a pulse oximeter (Veterinary Monitor, GT9000F, Guotenc, China), with the probe placed on the pinna or shaved tail root. Respiratory rate (RR) was determined by visually observing costo-abdominal movements, and mucous membrane color was assessed by observing the oral mucosa. Body temperature (BT) was measured rectally with a digital thermometer (Mesilife, Wuzi, China). To observe changes in BT, no external heating source was used; however, the animals were placed on a sponge pad.

Blood samples were collected for hematological analysis at baseline (t = -30 min), at 15, 30, 60, and 120 min, and at 24 hours (h) post-anesthesia. The samples were analyzed immediately after collection for a complete blood count using a hematology analyzer (Abacus Junior Vet 5, Diatron, Hungary).

Also lying on the side and standing up, responses to pain and reflex tests, chewing reflexes, quality of anesthesia induction, quality of surgical anesthesia and analgesia, quality of awakening and intubation tolerance were evaluated. These evaluations were subjectively evaluated with a scoring system (TABLE I) created by modifying the criteria applied by different researchers [3, 4, 5]. Scoring was done as; good = 3 points, moderate = 2 points and poor = 1 point.

In order to determine the quality and duration of surgical anesthesia and analgesia, the response to pinna compression and pedal retraction in the interdigital region between the fingers were evaluated. Scoring of the intubation procedure was subjectively evaluated by modifying the criteria applied [5].

TABLE I

Evaluation criteria for the quality of anesthesia induction, the quality of surgical anesthesia and analgesia, the quality of awakening, and the ease of intubation

Good = 3 points	Moderate = 2 points	Poor = 1 point
Induction Quality		
- Induction time < 3 min. - Sternal position or side lying. - No excitation. Good muscle relaxation. - No response to pain and reflex tests	- Induction time 3-5 min. - There is mild excitation - There is an attempt to get up after lying on the side. - Muscle relaxation is poor. - There is a mild response to pain and reflex tests.	- Induction time > 5 min - Excitation was evident. Did not lie on his side. - Muscle relaxation was weak. He made a sound. - There was a clear response to pain tests.
Quality of Surgical Anesthesia and Analgesia		
- No response to pain and reflex tests	- Reflex or localized muscle twitching, but no gross body movement.	- Distinct response to pain experiments.
	- Slight movement of head/legs.	- Noticeable movements throughout the body. - Withdrawal of legs. - Head liftling. - Chewing movements and vocalization. - Jaw tone is not completely lost. - Opening and closing of eyelids.
Quality of Awakening		
- It can walk with slight ataxia.	- Ataxia is noticeable while standing and walking. - Mild excitation is present.	- It cannot stand. - Struggling and thrashing on the ground were pronounced and prolonged. - Excitation is evident.
Ease/Comfort of Intubation		
< 2 attempts, < 2 min	< 4 attempts, < 5 min	> 4 attempts, > 5 min

Statistical analysis

Differences between groups were analyzed at each sampling time, with sampling time kept constant and groups considered as variables for clinical parameters and hematological parameters. Normality was assessed using the Shapiro-Wilk test. If the data were normally distributed, a one-way ANOVA was applied. In cases where significant differences were found between groups, the Tukey test was used if variances were homogeneous, whereas the Dunnett T3 test was applied when variances were not homogeneous. For non-parametric distributions, the Kruskal-Wallis test was used. If a significant difference was detected, pairwise comparisons were conducted using the Mann-Whitney U test. For within-group comparisons of hematological parameters, if the data followed a normal distribution, a Repeated Measures Analysis of Variance (RMANOVA) was conducted. If Mauchly's sphericity assumption was met, the Mauchly test was applied; otherwise, the Wilks-Lambda test was used. If the data were not normally distributed, the Friedman test was applied. If significant differences were observed in the Friedman test, pairwise comparisons were performed using the Wilcoxon test. The data obtained in this presented study were shown as mean value ± standard error (Mean ± SE). The difference was recorded as significant when $P \leq 0.05$. All statistical analyses were performed in a computer environment using the Minitab v11.0.

RESULTS AND DISCUSSION

All anesthetic combinations were used at the indicated doses in all groups and a safe general anesthesia was created without causing mortality. No significant findings were observed regarding complications (apnea and apneustic breathing) that may occur during and after anesthesia induction. However, no mortality or complications were observed on rabbits up to 24 h after the applications. Statistical analysis was conducted using One-way ANOVA for parametric physiological data and the Kruskal-Wallis test for non-parametric scoring data.

The cardiopulmonary effects of the anesthetic combinations for each group are presented in TABLE II, while the clinical effects are detailed in TABLE III.

It was observed that the HR was significantly lower in the Me-K-S group compared to the other groups ($P < 0.05$) at all sampling intervals. Specifically, HR decreased from baseline 230.6 ± 9.9 to 167.1 ± 12.7 at the 10th min, and this significant decrease persisted even at the 120th min (147.1 ± 16.4). Conversely, in the P-S group, an increase in HR was observed, rising from baseline 236.6 ± 14.0 to 256.0 ± 7.5 at the 10th min. The S group maintained high HR values without significant alteration 280.5 ± 15.5 at baseline vs 289.8 ± 9.3 at 10 min (TABLE II and FIG. 1).

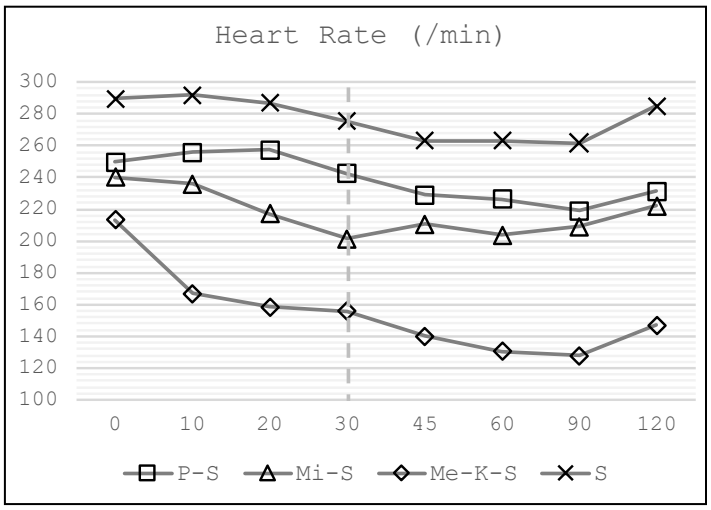

FIGURE 1. Effects of anesthesia protocols on heart rate. (Vertical dashed line indicates the 30th min when sevoflurane administration was discontinued. P-S: Propofol-Sevoflurane; Mi-S: Midazolam-Sevoflurane; Me-K-S: Medetomidine-Ketamine-Sevoflurane; S: Sevoflurane only).

Alpha-2 agonists are known to cause significant bradycardia, whereas propofol administration has been associated with increases in HR. Specifically, an elevation in HR has been documented up to 10 min following propofol administration [7]. Similarly, sevoflurane has also been reported to increase HR [8]. Although in this presented study, sevoflurane alone did not significantly alter HR, the increase observed in the P-S group continued for 20 min post-administration due to the effects of propofol, aligning with previously reported findings [7].

In animals administered only sevoflurane, the initial HR increase observed during the first 10 min after gas administration was attributed to rabbits' reactions to sevoflurane, such as convulsions. HR returned to normal progression as induction commenced (t = 10). Although previous reports indicate that sevoflurane alone may induce bradycardia during apneic respiratory periods, this was not observed due to HR measurements being recorded only at specified intervals in this presented study.

Also, when comparing groups in terms of HR, the Me-K-S group exhibited significantly lower HR values compared to other groups at all sampling intervals following injection. This decrease remained stable during sevoflurane administration after induction, with even more pronounced reductions noted after the termination of sevoflurane. These observations suggest that the α2-agonist medetomidine's effects were partially buffered by sevoflurane and Ketamine. However, following sevoflurane cessation and potential diminishing effects of Ketamine, the dominant α2-agonistic bradycardia re-emerged.

Regarding RR, significant decreases were observed in all groups compared to their control values during the inhalation anesthesia (10–30 min) ($P < 0.05$). The Me-K-S group showed a substantial reduction, decreasing from a baseline of 89.5 ± 7.9 to 36.1 ± 3.9 at the 30th min of anesthesia. Following the separation of rabbits from the anesthesia device, recovery patterns varied significantly. While the P-S and S groups returned to near-baseline levels rapidly (88.0 ± 9.6 and 77.3 ± 4.4, respectively, at the 60th min), the RR in the Me-K-S group remained significantly lower than the other groups (56.4 ± 4.0 at the 60th min) ($P < 0.05$) (TABLE II and FIG. 2).

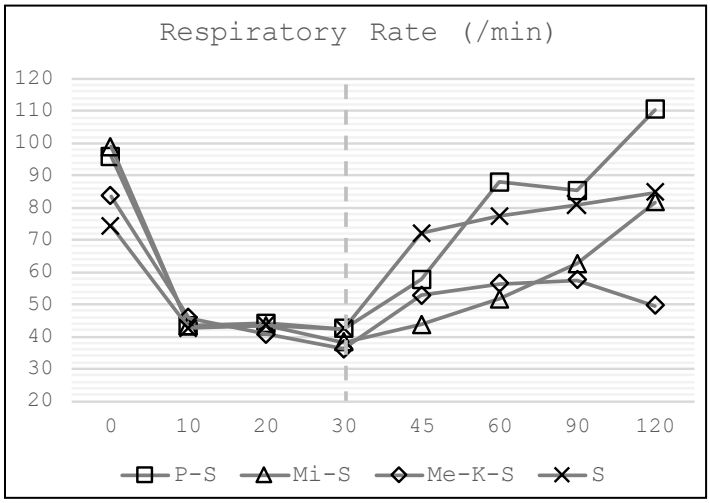

FIGURE 2. Effects of anesthesia protocols on respiratory rate. Vertical dashed line indicates the 30th min when sevoflurane administration was discontinued. P-S: Propofol-Sevoflurane; Mi-S: Midazolam-Sevoflurane; Me-K-S: Medetomidine-Ketamine-Sevoflurane; S: Sevoflurane only.

The RR exhibited a significant decrease following induction with sevoflurane across all groups. This decrease was observed immediately from the onset of mask application in the S group and was associated with apneic breathing [9]. The most pronounced reduction occurred in the Me-K-S group, likely due to the synergistic respiratory depressant effects of sevoflurane combined with medetomidine and ketamine. Upon cessation of Sevoflurane administration and removal of animals from the anesthesia machine, RR recovery commenced in all groups and rapidly reached baseline values in the P-S and Sevoflurane-only groups.

This rapid recovery indicated that the respiratory depressive effects of propofol and sevoflurane dissipated quickly. However, in the Me-K-S group, although recovery began, it did not return to initial levels, suggesting prolonged respiratory depression from premedication.

All groups showed significant decreases in the first 60 min of anesthesia for BT ($P < 0.05$). However, their recovery patterns differed considerably after this. Although the other groups gradually increased BT toward the 120th min, progressive BT decreases were recorded within the Me-K-S group. Specifically, BT within the Me-K-S group decreased from 39.2 ± 0.1 at baseline to 35.8 ± 0.7 at the 120th min. The Me-K-S group was therefore significantly lower than the others at both the 90th and 120th min ($P < 0.05$) (TABLE II and FIG. 3).

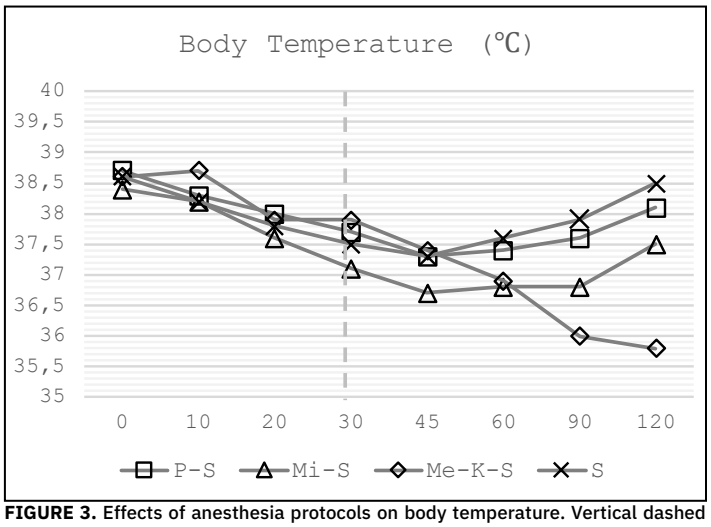

FIGURE 3. Effects of anesthesia protocols on body temperature. Vertical dashed line indicates the 30th min when sevoflurane administration was discontinued. P-S: Propofol-Sevoflurane; Mi-S: Midazolam-Sevoflurane; Me-K-S: Medetomidine-Ketamine-Sevoflurane; S: Sevoflurane only)

Respiratory depression induced by medetomidine in rabbits may lead to complications including hypotension, hypoxemia, hypercapnia, bradycardia, respiratory acidosis, and consequential hypothermia [10, 11]. In this study, a general decrease in BT was observed until the 45th–60th min of anesthesia across all groups, consistent with anesthesia-induced thermoregulatory depression.

However, while other groups demonstrated recovery thereafter, the Me-K-S group demonstrated a prolonged decrease lasting up to 120 min. Therefore, active warming and monitoring of BT alongside respiratory parameters are critical in rabbits anesthetized using this combination to prevent severe hypothermia.

Regarding SpO^2 generally remained stable or increased slightly during the inhalation anesthesia period (10–30 min) due to the administration of 100 % oxygen. By the 120th min, SpO^2 values in all groups returned to baseline levels (TABLE II).

A relative decrease in SpO_2 values was observed in the Me-K-S group upon removal from the anesthesia machine. Although no significant differences in SpO_2 values were observed among groups during sevoflurane administration, slight increases were noted, likely due to sevoflurane delivery in 100 % oxygen.

Crucially, SpO^2 values returned to baseline levels by the 120th min without any additional intervention. This recovery pattern indicates that the temporary respiratory effects of the combinations are reversible and do not compromise the clinical safety of the protocol, as the animals successfully regained normal oxygenation saturation during the recovery period.

Throughout the 30 min anesthesia, the Me-K-S group consistently demonstrated superior surgical anesthesia/analgesia quality (P < 0.05). Specifically, at the 20th min, the Me-K-S group sustained a high-quality score of 2.75 ± 0.5 (out of 3), which was significantly higher than the P-S (1.63 ± 0.5) and Sevoflurane-only (1.50 ± 0.5) groups (P < 0.05). This persisted at the 30th min as well (2.13 ± 0.4), indicating that the Me-K-S combination maintained a deeper anesthetic plane during the entire inhalation procedure (TABLE II).

Although no surgical intervention was performed to directly assess the quality of surgical anesthesia and analgesia in this study, efficacy was evaluated based on responses to reflex examinations and standardized pain stimuli. The superior anesthesia quality scores observed in the Me-K-S group are consistent with previous studies involving medetomidine and ketamine in rabbits [3, 10, 11, 12]. These studies suggest that Me-K-S provides sufficient surgical anesthesia and analgesia for invasive procedures, which aligns with the higher reflex suppression scores observed in our Me-K-S group compared to the other protocols.

TABLE II

Mean values of heart rate, respiratory rate, body temperature, oxygen saturation measurements, and quality of surgical anesthesia/analgesia for the groups

Control		10 min	20 min	30 min	60 min	90 min	120 min
Heart Rate (/min)							
P-S	236.6 ± 14.0^{ab}	256.0 ± 7.5^{a}	257.3 ± 10.1^{a}	242.6 ± 7.6^{a}	226.4 ± 5.1^{ad}	219.1 ± 7.5^{a}	231.2 ± 10.9^{a}
Mi-S	231.1 ± 8.5^{a}	236.0 ± 10.9^{a}	217.3 ± 10.8^{b}	201.3 ± 10.1^{b}	203.7 ± 9.6^{a}	209.3 ± 7.2^{a}	222.2 ± 5.4^{a}
Me-K-S	230.6 ± 9.9^{a}	167.1 ± 12.7^{b}	158.6 ± 7.4^{c}	156.0 ± 6.9^{c}	130.6 ± 9.4^{b}	128.0 ± 13.4^{b}	147.1 ± 16.4^{b}
S	280.5 ± 15.5^{b}	291.8 ± 11.6^{c}	286.8 ± 10.1^{d}	275.5 ± 9.8^{d}	263.0 ± 13.5^{d}	261.1 ± 10.3^{c}	285.0 ± 8.1^{c}
Respiratory Rate (/min)							
P-S	102.6 ± 10.4	43.3 ± 3.9	44.0 ± 3.6	42.4 ± 3.0	88.0 ± 9.6^{a}	85.3 ± 10.7^{ab}	110.4 ± 8.8^{b}
Mi-S	96.3 ± 12.8	43.1 ± 2.4	43.3 ± 3.1	38.2 ± 3.2	51.6 ± 5.2^{b}	62.6 ± 8.3^{ab}	81.6 ± 15.6^{ac}
Me-K-S	89.5 ± 7.9	45.8 ± 4.9	40.7 ± 4.2	36.1 ± 3.9	56.4 ± 4.0^{c}	57.5 ± 5.2^{b}	49.5 ± 4.4^{a}
S	74.6 ± 4.1	42.6 ± 1.6	43.5 ± 1.4	42.4 ± 1.3	77.3 ± 4.4^{ac}	80.8 ± 3.9^{a}	84.8 ± 2.0^{c}
Body Temperature (°C)							
P-S	39.2 ± 0.1	38.3 ± 0.1	38.0 ± 0.2	37.7 ± 0.2	37.4 ± 0.2	37.6 ± 0.2^{ab}	38.1 ± 0.2^{ab}
Mi-S	38.6 ± 0.1	38.2 ± 0.1	37.6 ± 0.1	37.1 ± 0.2	36.8 ± 0.2	36.8 ± 0.2^{a}	37.5 ± 0.2^{a}
Me-K-S	39.2 ± 0.1	38.7 ± 0.2	37.9 ± 0.5	37.9 ± 0.3	36.9 ± 0.3	36.0 ± 0.5^{a}	35.8 ± 0.7^{a}
S	38.9 ± 0.1	38.2 ± 0.2	37.8 ± 0.2	37.5 ± 0.1	37.6 ± 0.1	37.9 ± 0.1^{b}	38.5 ± 0.1^{b}
SpO_2 (%)							
P-S	96.6 ± 0.4^{abc}	97.6 ± 0.2^{abc}	97.7 ± 0.3^{ab}	98.4 ± 0.2	96.3 ± 0.2^{a}	95.1 ± 0.8^{a}	96.8 ± 0.3^{ab}
Mi-S	96.4 ± 0.7^{abc}	98.2 ± 0.3^{b}	98.2 ± 0.4^{ab}	97.5 ± 0.6	96.7 ± 0.3^{ac}	95.3 ± 1.3^{ab}	96.0 ± 0.4^{a}
Me-K-S	95.1 ± 0.6^{cb}	95.6 ± 1.0^{c}	96.3 ± 1.1^{a}	94.3 ± 1.5	89.1 ± 1.4^{b}	93.4 ± 0.8^{a}	95.4 ± 0.6^{a}
S	97.4 ± 0.2^{ab}	98.4 ± 0.2^{b}	98.6 ± 0.1^{b}	98.2 ± 0.2	97.3 ± 0.2^{c}	97.2 ± 0.3^{b}	97.5 ± 0.2^{b}
SpO_2 (%)							
P-S	96.6 ± 0.4^{abc}	97.6 ± 0.2^{abc}	97.7 ± 0.3^{ab}	98.4 ± 0.2	96.3 ± 0.2^{a}	95.1 ± 0.8^{a}	96.8 ± 0.3^{ab}
Mi-S	96.4 ± 0.7^{abc}	98.2 ± 0.3^{b}	98.2 ± 0.4^{ab}	97.5 ± 0.6	96.7 ± 0.3^{ac}	95.3 ± 1.3^{ab}	96.0 ± 0.4^{a}
Me-K-S	95.1 ± 0.6^{cb}	95.6 ± 1.0^{c}	96.3 ± 1.1^{a}	94.3 ± 1.5	89.1 ± 1.4^{b}	93.4 ± 0.8^{a}	95.4 ± 0.6^{a}
S	97.4 ± 0.2^{ab}	98.4 ± 0.2^{b}	98.6 ± 0.1^{b}	98.2 ± 0.2	97.3 ± 0.2^{c}	97.2 ± 0.3^{b}	97.5 ± 0.2^{b}
Quality of Surgical Anesthesia/Analgesia*							
P-S	NA	2,25 ± 0,5$^{x,\ a}$	1,63 ± 0,5^{x,b}	1,62 ± 0,5^{x,b}	1,00 ± 0,0$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$
Mi-S	NA	2,29 ± 0,5$^{xy,\ a}$	2,00 ± 0,0$^{y,\ ab}$	1,71 ± 0,5^{x,b}	1,14 ± 0,4$^{x,\ c}$	1,14 ± 0,4$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$
Me-K-S	NA	2,75 ± 0,5$^{xy\ a}$	2,75 ± 0,5$^{x,\ a}$	2,13 ± 0,4^{x,b}	1,38 ± 0,5$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$
S	NA	2,15 ± 0,5$^{x,\ a}$	1,50 ± 0,5^{y,b}	1,50 ± 0,5^{x,b}	1,00 ± 0,0$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$	1,00 ± 0,0$^{x,\ c}$

Means with different letters in the same row (a, b, c, d) and column (x, y) are statistically significantly different (P < 0.05).
* Evaluated based on the criteria in Table 1, good = 3 points, moderate = 2 points, and poor = 1 point.
Control values were recorded 30 min before the administration of pre-anesthetic/anesthetic drugs.
The 30th min marks the time point when sevoflurane administration was discontinued.
P-S: Propofol-Sevoflurane; Mi-S: Midazolam-Sevoflurane; Me-K-S: Medetomidine-Ketamine-Sevoflurane; S: Sevoflurane only.

While propofol is recognized as a short-acting hypnotic agent with limited analgesic properties [5, 9, 13], midazolam has been shown to produce excellent muscle relaxation and sedative-hypnotic effects in rabbits, particularly at higher doses [14, 15]. However, the dose of midazolam used in this presented study was significantly lower than the commonly reported. This lower dose, chosen to minimize cardiovascular and respiratory depression, likely resulted in insufficient hypnotic and analgesic depth, especially when not combined with other sedative/analgesic agents. Adequate surgical anesthesia and analgesia were not achieved in this group. Furthermore, the absence of a statistically significant difference between the groups at 90 and 120 min of anesthesia suggests that for prolonged surgical procedures, maintenance with inhalation anesthesia is recommended, and the need for additional anesthetic or analgesic support should be carefully evaluated through continuous monitoring.

The shortest anesthesia induction time was recorded in the P-S group (2.50 ± 0.45), followed by the Me-K-S (3.18 ± 0.40) and Mi-S (3,75 ± 0,83), while the S had the significantly slowest induction (4.25 ± 0.45) (P < 0.05). On the other hand, the quality of anesthesia induction was poorest in the P-S group with a score of 1.70 ± 0.52 (out of 3). This was significantly lower than the Me-K-S group, which achieved the best induction quality score (2.90 ± 0.48) (P < 0.05).

During airway management, intubation was significantly more difficult in the Mi-S group (1.15 ± 0.32) (P < 0.05). Recovery parameters also varied significantly; the Me-K-S group exhibited the longest time to onset of chewing reflex (75.30 ± 15.6) and the longest righting reflex duration (84.00 ± 8.10). Consequently, the quality of awakening was observed to be quite poor in the Me-K-S group (1.35 ± 0.51) compared to the P-S group (2.70 ± 0.32) (P < 0.05) (TABLE III).

In experimental studies and clinical applications, it is crucial to provide safe and effective anesthesia in rabbits. Inhalation anesthesia is considered the safest method to achieve balanced and controlled anesthesia, especially during prolonged procedures. However, due to the anatomical structure of rabbits, equipment cost, and practical difficulties, intubation techniques for inhalation anesthesia are not widely employed [16, 17, 18, 19]. Premedication agents used to facilitate intubation should induce muscle relaxation and loss of pharyngolaryngeal reflexes, enabling easier mouth opening. In this presented study, intubation was performed following the administration of injectable induction agents. Ease of intubation was observed to be reduced in the Mi-S group, suggesting that the chosen premedication dose was inadequate.

Although intubation was achievable, insufficient muscle relaxation and incomplete loss of pharyngolaryngeal reflexes complicated the procedure. It is important to emphasize that forcing intubation in a patient without adequate sedation, muscle relaxation, and loss of pharyngolaryngeal reflexes can be highly stressful and carries significant risks, such as laryngeal trauma and edema.

Conversely, intubation was easier in groups receiving medetomidine-ketamine and propofol as premedication agents, particularly in the Me-K-S group. The time to the onset of the chewing reflex (extubation time) and the righting reflexes were notably prolonged in the Me-K-S group compared to other groups.

Previous studies have indicated that shorter recovery periods positively influence anesthesia recovery quality [3]. Consequently, in this presented study, recovery quality was ranked from good to poor as follows: P-S, Mi-S, S, and Me-K-S. The Me-K-S combination provided deeper and prolonged suppression of laryngeal and pharyngeal reflexes, facilitating intubation and inhalation anesthesia but negatively impacting recovery quality.

Anesthesia induction times were compared in this presented study, the fastest induction was observed in the P-S group, while the slowest was recorded in the sevoflurane-only group. This finding aligns with the well-documented properties of propofol, which has high lipid solubility, a rapid onset of action, and is a

potent hypnotic agent capable of inducing anesthesia within a very short period [9, 13].

In contrast, induction took significantly longer in the S group, likely due to the apneic effects of the inhalant agent [20], which hindered adequate agent uptake. This observation highlights the necessity of premedication not only to enhance the quality of anesthesia and analgesia but also to accelerate the induction process.

When time-dependent changes in hematological parameters after anesthesia were analyzed, no significant differences were found between the groups. However, significant time-dependent changes were observed in WBC counts in Mi-S and Me-K-S. Regarding hemoglobin levels, significant within-group changes were detected only in Me-K-S. Similarly, significant within-group changes in platelet counts were observed only in Mi-S.

TABLE III
Mean values of clinical anesthesia assessment parameters for the groups

	P-S	Mi-S	Me-K-S	S
Anesthesia Induction Time (min)	2,5 ± 0,45^{b}	3,75 ± 0,83^{ab}	3,18 ± 0,40^{ab}	4,25 ± 0,45^{a}
Anesthesia Sedation/Induction Quality*	1,70 ± 0,52	2,30 ± 0,52	2,90 ± 0,48	2,2 ± 0,52
Ease of Intubation*	2,15 ± 0,56	1,15 ± 0,32	2,75 ± 0,50	NA
Chewing Reflex Onset Time (Extubation Time) (min)	41,90 ± 4,15^{a}	42,40 ± 3,48^{a}	75,30 ± 15,6^{b}	47,70 ± 3,12^{a}
Righting Reflex Duration (min)	45,30 ± 1,80^{a}	46,30 ± 1,30^{a}	84,00 ± 8,10^{b}	49,60 ± 3,20^{a}
Quality of Awakening*	2,70 ± 0,32^{b}	2,50 ± 0,53^{ab}	1,35 ± 0,51^{a}	2,90 ± 0,35

Means with different letters in the same row (a, b, c, d) and column (x, y) are statistically significantly different ($P < 0.05$). * Evaluated based on the criteria in Table 1, good = 3 points, moderate = 2 points, and poor = 1 point.P-S: Propofol-Sevoflurane; Mi-S: Midazolam-Sevoflurane; Me-K-S: Medetomidine-Ketamine-Sevoflurane; S: Sevoflurane only.

Sedation and anesthesia can induce hematological variations in rabbits. It has been previously reported that a significant reduction in WBC counts in sedated rabbits compared to the non-sedated group [21]. Similarly, a significant decrease reported in RBC counts at the first and second hours and an increase in WBC counts at the first, second, and 24th h after the administration of acepromazine, midazolam, and ketamine [22].

On the other hand, no significant differences also reported in hematological values in rabbits anesthetized with xylazine-ketamine, xylazine-tiletamine-zolazepam, or Tiletamine-Zolazepam protocols [23]. Although previous studies have reported varying degrees of hematological changes following sedation and anesthesia, the findings remain inconsistent. In the present study, while some significant variations in hematological parameters were observed, these changes do not appear to be of clinical significance.

This presented study possesses strengths and limitations. Primarily, the comparison of multiple anesthetic protocols using objective cardiopulmonary and clinical parameters enabled a comprehensive assessment of both efficacy and safety.

While time-dependent monitoring of physiological variables provided valuable insight into the dynamic effects of each protocol. Nevertheless, as limitation the absence of an actual surgical procedure precluded direct assessment of intraoperative analgesia and surgical anesthesia depth, relying instead on reflex-based scoring methods that may introduce subjectivity. However, there are several limitations.

First, although the sample size (n = 10 per group) was sufficient to detect statistical differences in major physiological parameters, it may be limited in detecting rare adverse events. Second, the evaluation of anesthesia depth and quality relied on reflex-based scoring systems. Although standardized, these methods may cause a degree of observer bias compared to objective electroencephalographic monitoring. Third, this study was conducted on healthy subjects; therefore, the hemodynamic depression observed in the Me-K-S group might be more profound and clinically significant in compromised patients.

The observation period was limited to the acute perioperative phase. Therefore, potential long-term complications that were not reported in this study, such as tracheal stenosis resulting from subclinical intubation trauma or ileus associated with the prolonged effects of alpha-2 agonists, could not be evaluated. Future studies with longer follow-up periods and larger population including actual surgical procedures are warranted to further refine these anesthetic protocols.

CONCLUSION

Based on the superior analgesic depth observed in this study and supported by previous literature, the Me-K-S combination may be considered a suitable option for rabbits undergoing complex procedures. However, it was also associated with prolonged recovery times, deeper cardiopulmonary depression, and increased risk of hypothermia. Propofol anesthesia allowed for rapid induction and smoother recovery, yet insufficient analgesic depth. The use of low-dose midazolam alone proved insufficient for surgical anesthesia, highlighting the importance of appropriate dosing and multimodal anesthesia approaches. Although inhalation anesthesia remains important for prolonged procedures, challenges in airway management in rabbits underline the importance of premedication and agent selection. Further studies involving actual surgical interventions and long-term follow-up are warranted to refine anesthetic protocols for use in rabbits.

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Conflicting interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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