

Comparative evaluation of clinical and cardiovascular effects of different doses and administration routes of ketamine-medetomidine combination in rats

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Summary

This study aimed to evaluate the clinical and cardiopulmonary effects of different ketamine-medetomidine combinations administered via subcutaneous (SC) and intraperitoneal (IP) routes in rats and to contribute to the development of safe injectable anesthesia protocols. Forty-eight male rats (*Rattus norvegicus*) were randomly assigned to six experimental groups according to anesthetic dose and administration route. Induction time, total sleep time, surgical anesthesia time, heart rate, respiratory rate, rectal temperature, peripheral oxygen saturation (SpO₂), and anesthetic reflexes were evaluated. Statistical analyses were performed using repeated measures ANOVA followed by Tukey's post hoc test, with significance set at $P < 0.05$. All anesthetic protocols successfully induced anesthesia without mortality or severe complications. IP administration produced significantly shorter induction times than SC administration ($P < 0.05$), with the shortest induction time observed in Group 4 (1.40 ± 0.21 min). Durations of total anesthesia and surgical anesthesia were significantly longer in Groups 1 and 3 ($P < 0.05$). Heart rate, respiratory rate, rectal temperature, and SpO₂ values significantly decreased after anesthetic administration in all groups ($P < 0.05$). Cardiopulmonary depression and hypothermia were more pronounced in higher-dose and IP administration groups. Adequate surgical anesthesia was confirmed by loss of pedal withdrawal and palpebral reflexes. Ketamine-medetomidine combinations administered through both SC and IP routes provided reliable injectable anesthesia in rats. IP administration resulted in faster induction and deeper anesthesia, whereas higher doses produced prolonged anesthesia with greater cardiopulmonary depression. External thermal support and oxygen supplementation are recommended during prolonged anesthetic procedures.

Keywords: ketamine, medetomidine, rat anesthesia, cardiopulmonary parameters, injectable anesthesia

Rats (*Rattus norvegicus*) are among the most widely used laboratory animal species in biomedical and experimental research because of their well-characterized physiological and pharmacological properties, ease of handling, and suitability for experimental manipulation (4, 6). Reliable anesthesia protocols are essential not only for facilitating experimental procedures, but also for minimizing stress, pain, and physiological disturbances that may affect research outcomes. Due to their high metabolic rate and sensitivity to anesthetic agents, rats are particularly susceptible to complica-

tions, such as hypothermia, respiratory depression, and cardiovascular alterations during anesthesia (6, 8). Therefore, careful selection of anesthetic agents and appropriate dosing are critical for both animal welfare and the reliability of experimental data.

Injectable anesthesia is commonly preferred in rodents because it is practical, economical, and does not require specialized inhalation equipment. Among injectable anesthetic agents, ketamine is one of the most frequently used dissociative anesthetics in laboratory animals. Ketamine produces anesthesia and analgesia

primarily through antagonism of N-methyl-D-aspartate (NMDA) receptors and is known to preserve cardiovascular function better than many other anesthetic agents (13). However, ketamine alone may provide insufficient muscle relaxation and inadequate anesthetic depth, which may lead to undesirable excitatory effects during recovery.

To overcome these limitations, ketamine is commonly combined with alpha-2 adrenergic agonists, such as medetomidine. Medetomidine provides sedation, analgesia, and muscle relaxation through stimulation of central alpha-2 adrenergic receptors, thereby improving anesthetic quality and reducing the required ketamine dose (7, 14). Furthermore, atipamezole, a specific antagonist of medetomidine, is available for rapid and controlled reversal of anesthesia when necessary. Despite these advantages, medetomidine may induce significant cardiovascular effects, including bradycardia and alterations in blood pressure, which necessitate careful monitoring.

The route of administration is another important factor influencing the onset, duration, and depth of anesthesia. Intraperitoneal administration is widely used in rats because of its technical simplicity and rapid systemic absorption, whereas subcutaneous administration may provide slower absorption and a more gradual anesthetic induction (6, 20). Comparative evaluation of different administration routes may therefore contribute to the development of safer and more predictable anesthetic protocols.

Previous studies have reported that ketamine-medetomidine combinations provide effective and safe anesthesia in rats at ketamine doses ranging from 40 to 100 mg/kg and medetomidine doses between 0.1 and 0.5 mg/kg (4, 6, 7). Therefore, the present study aimed to compare the clinical and cardiovascular effects of different ketamine-medetomidine dose combinations administered via subcutaneous and intraperitoneal routes in rats and to contribute to the optimization of injectable anesthesia protocols for laboratory animal practice.

Although ketamine-medetomidine combinations have been extensively used for injectable anesthesia in laboratory rodents, previous studies have focused primarily on single-dose protocols or a single route of administration. Comparative information regarding different dose combinations administered via both subcutaneous and intraperitoneal routes under identical experimental conditions remains limited. Therefore, the present study was designed to comprehensively compare anesthetic induction, surgical anesthetic duration, recovery characteristics, and cardiopulmonary responses following different ketamine-medetomidine dose combinations administered through these two commonly used routes in rats. The findings are expected to provide practical guidance for selecting appropriate anesthetic protocols according to the requirements of various experimental procedures.

Material and methods

Animals. This study was designed to evaluate the clinical and cardiovascular effects of different doses and administration routes of ketamine-medetomidine combinations in rats. A total of 48 adult male rats (*Rattus norvegicus*), weighing 250-350 g, were used in the study. The animals were obtained from the Experimental Animal Unit of Aydın Adnan Menderes University Faculty of Veterinary Medicine.

The sample size ($n = 8$ per group) was determined based on ethical considerations for laboratory animal use, previous studies employing similar experimental designs, and the principle of reduction in accordance with the 3Rs. This number was considered sufficient to detect clinically meaningful differences while minimizing the number of animals used.

Before the experimental procedures, all animals underwent a 7-day acclimatization period. During this period, their general health status was evaluated through clinical examination, and only clinically healthy animals were included in the study.

The rats were housed under standardized environmental conditions at a temperature of 20-24°C, relative humidity of 40-60%, and a 12 h light/12 h dark photoperiod. Sterile wood shavings were used as bedding material, and cages were cleaned daily. Standard commercial rat chow and water were provided *ad libitum*. All housing and experimental procedures were conducted in accordance with laboratory animal welfare guidelines. Following acclimatization, the animals were randomly assigned to six experimental groups ($n = 8$ per group) using a simple randomization procedure.

Experimental design. The animals were randomly divided into six experimental groups of eight male rats each:

Group 1: SC medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 2: SC medetomidine (0.75 mg/kg) + ketamine (75 mg/kg)

Group 3: SC medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

Group 4: IP medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 5: IP medetomidine (0.75 mg/kg) + ketamine (75 mg/kg)

Group 6: IP medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

The anesthetic combinations were prepared according to the designated doses and administered as a single injection via the SC or IP route (4, 5, 7). Baseline measurements were recorded before anesthetic administration.

Anesthetic evaluation. *Induction time* was defined as the interval between administration of the anesthetic combination and loss of the righting reflex. *Surgical anesthesia duration* was defined as the period between loss and return of the pedal withdrawal and auricular reflexes, indicating an adequate surgical plane of anesthesia. *Total sleep time* was defined as the interval between loss and recovery of the righting reflex. These parameters were used to evaluate anesthetic onset, depth, and duration in all experimental groups (10, 13).

Clinical and cardiovascular evaluation. Clinical and cardiovascular parameters were recorded immediately after anesthetic administration (0 min) and at 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min. Respiratory rate was determined by observing thoracic movements over 1 min. Heart rate was measured with a physiological monitor. Body temperature was measured rectally with a digital thermometer, while peripheral oxygen saturation (SpO₂) was measured with a pulse oximeter. Pedal withdrawal reflex was evaluated every 5 min by applying pressure to the interdigital skin using forceps; limb withdrawal, vocalization, or muscle contraction were considered positive responses. Palpebral reflex was assessed by observing eyelid contraction following stimulation. To minimize circadian variations, all anesthetic procedures were performed between 1 p.m. and 2 p.m.

During anesthesia, all animals were placed on a thermostatically controlled heating pad to maintain body temperature and received supplemental oxygen throughout the anesthetic period. These supportive measures were standardized across all experimental groups.

Statistical analysis. The statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean $\pm$ standard deviation (SD). The normality of data distribution was evaluated using the Kolmogorov-Smirnov test. Temporal and intergroup comparisons were analyzed by two-way repeated measures analysis of variance (ANOVA). When significant differences were detected, Tukey's post hoc multiple comparison test was applied. Non-parametric data were analyzed using the Friedman test followed by Mann-Whitney U test for pairwise comparisons. Differences were considered statistically significant at $P < 0.05$. Different lowercase superscript letters indicate statistically significant intergroup differences, whereas different uppercase superscript letters indicate statistically significant intragroup temporal differences. An asterisk (*) denotes statistically significant differences compared with baseline values within the same group.

Tab. 1. Induction time, total sleep time, and surgical anesthesia time in experimental groups. Values are expressed as mean $\pm$ standard deviation (SD). Different superscript letters (a-d) within the same column indicate statistically significant differences between groups ($P < 0.05$)

Group	Induction time (min)	Total sleep time (min)	Surgical anesthesia time (min)	Statistical significance
Group 1	8.84 $\pm$ 0.98 ^a	451.00 $\pm$ 13.80 ^a	124.38 $\pm$ 4.14 ^a	$P < 0.05$
Group 2	7.67 $\pm$ 0.53 ^b	366.12 $\pm$ 25.40 ^b	94.00 $\pm$ 6.12 ^b	$P < 0.05$
Group 3	8.54 $\pm$ 3.15 ^a	462.12 $\pm$ 15.84 ^a	110.00 $\pm$ 44.73 ^a	$P < 0.05$
Group 4	1.40 $\pm$ 0.21 ^c	405.88 $\pm$ 14.74 ^c	128.50 $\pm$ 5.93 ^c	$P < 0.05$
Group 5	2.08 $\pm$ 0.16 ^d	329.62 $\pm$ 17.34 ^d	92.62 $\pm$ 5.78 ^d	$P < 0.05$
Group 6	2.12 $\pm$ 0.24 ^d	349.50 $\pm$ 23.41 ^d	94.12 $\pm$ 6.49 ^d	$P < 0.05$

Explanations: ANOVA results: Induction time ($P = 0.0000$), total anesthesia time ($P = 0.0000$), and surgical anesthesia time ($P = 0.0004$) showed statistically significant differences between groups.

Group 1: SC medetomidine (1 mg/kg) + ketamine (100 mg/kg)
 Group 2: SC medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)
 Group 3: SC medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)
 Group 4: IP medetomidine (1 mg/kg) + ketamine (100 mg/kg)
 Group 5: IP medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)
 Group 6: IP medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

Ethical approval. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals. At the end of the study, all animals were monitored until complete recovery from anesthesia and were maintained for future non-terminal experimental use. This study was conducted with the approval of the Aydın Adnan Menderes University Local Ethics Committee for Animal Experiments (ADÜ-HADYK), dated 30.04.2026 and numbered 64583101/2026/074.

Results and discussion

Induction time, total sleep time, and surgical anesthesia time. The effects of different ketamine-medetomidine combinations and administration routes on induction time, total sleep time, and surgical anesthesia time are presented in Table 1. Statistically significant differences were observed between experimental groups for the selected anesthetic variables ($P < 0.05$). Intraperitoneal administration resulted in significantly shorter induction times compared with subcutaneous administration. The shortest induction time was recorded in Group 4 (1.40 ± 0.21 min), followed by Groups 5 and 6, whereas the longest induction times were observed in Groups 1 and 3. These findings clearly demonstrate that the route of administration substantially influenced the onset of anesthesia. Total sleep time was significantly longer in Groups 1 and 3 than in Groups 2, 5, and 6, while surgical anesthesia duration was significantly prolonged in Groups 1 and 4. These results indicate that both anesthetic dose and administration route contributed to the duration and depth of anesthesia.

The markedly shorter induction times following intraperitoneal administration are consistent with previous reports describing the pharmacokinetic advantages of this route in rodents. Intraperitoneal administration

leads to rapid absorption of anesthetic agents through the highly vascularized peritoneal surface, resulting in faster systemic distribution and earlier onset of anesthetic effects (6, 20). In contrast, subcutaneous administration is characterized by slower absorption kinetics because drug uptake depends largely on local tissue perfusion. Consequently, anesthetic agents administered subcutaneously require a longer period to reach effective plasma concentrations, which explains the prolonged induction periods observed in Groups 1-3.

The present findings are in agreement with previous studies evaluating injectable anesthetic protocols in laboratory rodents. It has been reported that ketamine-medetomidine combinations provide reliable anesthesia with rapid induction and

sufficient surgical depth when administered through routes allowing efficient systemic absorption (6, 14). Similarly, studies investigating balanced anesthetic combinations have demonstrated that administration route significantly influences induction characteristics and anesthetic quality (10, 11). The rapid induction observed particularly in Group 4 suggests that intraperitoneal administration of ketamine and medetomidine may be advantageous in experimental settings where rapid anesthetic onset is desirable.

Anesthetic duration was also strongly influenced by both ketamine dose and medetomidine concentration. The groups receiving ketamine at 100 mg/kg generally exhibited longer total anesthesia and surgical anesthesia durations than the lower-dose groups. Ketamine produces dissociative anesthesia through antagonism of N-methyl-D-aspartate (NMDA) receptors, whereas medetomidine induces sedation, analgesia, and muscle relaxation through stimulation of central α_2 -adrenoceptors (15, 16). The interaction between these pharmacological mechanisms results in synergistic anesthetic effects, thereby prolonging anesthetic depth and duration. This synergism likely explains the prolonged anesthesia observed in Groups 1, 3, and 4.

The particularly long total anesthesia duration observed in Group 3 is noteworthy because this group received a lower medetomidine dose but a higher ketamine dose. This finding suggests that ketamine dosage may exert a stronger influence on maintenance of anesthesia than medetomidine dose alone. Previous investigations have similarly reported that increasing ketamine dosage significantly prolongs anesthetic duration and recovery time in rodents (4, 6). In contrast, animals receiving lower ketamine doses recovered more rapidly despite receiving comparable medetomidine doses.

The prolonged surgical anesthesia observed in Groups 1 and 4 may have practical advantages for experimental procedures requiring extended operating times. Surgical anesthesia exceeding 120 min may reduce the need for supplemental anesthetic administration during lengthy procedures and minimize handling-related stress. However, prolonged anesthesia may also increase the risk of hypothermia, respiratory depression, and cardiovascular compromise. Therefore, protocols producing longer anesthetic durations should be accompanied by appropriate physiological monitoring and supportive care measures.

Another notable finding was the difference between Groups 1 and 4, which received identical drug doses via different administration routes. Although Group 4 demonstrated substantially shorter induction times, surgical anesthesia duration remained comparable to that of Group 1. This observation suggests that intraperitoneal administration primarily affects the rate of drug absorption and anesthetic onset rather than the overall effectiveness of the anesthetic combination

once adequate systemic concentrations have been achieved.

Collectively, these findings indicate that both dosage and route of administration play critical roles in determining anesthetic performance in rats. Intraperitoneal administration provides a significant advantage in terms of rapid induction, whereas higher ketamine-containing protocols provide longer anesthetic duration and more sustained surgical anesthesia. Consequently, selection of the most appropriate ketamine-medetomidine protocol should be based on the expected duration of the procedure, required anesthetic depth, and availability of physiological monitoring throughout anesthesia.

Heart rate. Temporal changes in heart rate during anesthesia are presented in Table 2 and Figure 1A. Repeated measures ANOVA demonstrated significant intragroup and intergroup differences throughout the monitoring period ($P < 0.05$). Heart rate values decreased significantly following anesthetic administration in all experimental groups compared with baseline measurements. The greatest reductions were generally observed between 15 and 30 min after induction. In Group 1, heart rate decreased from 238 ± 39 beats/min at baseline to 175 ± 28 beats/min at 30 min. Similar reductions were observed in Group 2 (229 ± 35 to 181 ± 24 beats/min) and Group 3 (239 ± 33 to 196 ± 17 beats/min). Comparable decreases were also recorded in the IP administration groups. Although partial recovery was observed after 60 min, heart rate values remained significantly below baseline levels throughout most of the monitoring period.

The reduction in heart rate observed in all groups is consistent with the well-documented cardiovascular effects of medetomidine. Medetomidine is a highly selective α_2 -adrenergic receptor agonist that decreases sympathetic nervous system activity by stimulating central presynaptic α_2 -adrenoceptors. Activation of these receptors suppresses norepinephrine release within the central nervous system, resulting in reduced sympathetic tone, decreased cardiac stimulation, and subsequent bradycardia (16). This pharmacological mechanism explains the significant reductions in heart rate observed shortly after anesthetic administration in all experimental groups.

Previous studies have consistently demonstrated that medetomidine-containing anesthetic protocols induce marked decreases in heart rate in laboratory rodents. Stable but significantly reduced heart rates have been reported following administration of medetomidine-midazolam-butorphanol combinations in rats and other small laboratory animals (14, 17). Similar findings have also been described in studies evaluating balanced anesthetic protocols containing α_2 -adrenergic agonists, where cardiovascular depression was considered an expected pharmacological consequence of enhanced parasympathetic predominance and reduced sympathetic activity (10, 14, 16).

Tab. 2. Temporal changes in cardiopulmonary parameters during anesthesia

Group	Parameter	0 min	15 min	30 min	60 min	120 min	Intragroup p
Group 1	Heart rate	238 ± 39 ^{aA}	192 ± 26 ^{aB*}	175 ± 28 ^{aC*}	186 ± 31 ^{aBc*}	201 ± 42 ^{aB*}	< 0.05
	Respiratory rate	106 ± 19 ^{aA}	73 ± 18 ^{aB*}	78 ± 14 ^{aB*}	91 ± 16 ^{aC*}	96 ± 20 ^{aC*}	< 0.01
	Body temperature	37.4 ± 0.4 ^{aA}	35.2 ± 0.5 ^{aB*}	34.1 ± 0.6 ^{aC*}	33.4 ± 0.7 ^{aD*}	34.8 ± 0.6 ^{aC*}	< 0.001
	SpO ₂	95 ± 4 ^{aA}	91 ± 5 ^{aB*}	89 ± 5 ^{aB*}	92 ± 4 ^{aB*}	96 ± 3 ^{aA}	< 0.05
Group 2	Heart rate	229 ± 35 ^{aA}	198 ± 20 ^{aB*}	181 ± 24 ^{aC*}	191 ± 26 ^{aB*}	194 ± 37 ^{aB*}	< 0.05
	Respiratory rate	111 ± 22 ^{aA}	70 ± 16 ^{aB*}	74 ± 13 ^{aB*}	82 ± 15 ^{aC*}	89 ± 18 ^{aC*}	< 0.01
	Body temperature	37.5 ± 0.3 ^{aA}	35.0 ± 0.4 ^{aB*}	34.0 ± 0.5 ^{aC*}	33.2 ± 0.6 ^{aD*}	34.6 ± 0.5 ^{aC*}	< 0.001
	SpO ₂	96 ± 3 ^{aA}	92 ± 4 ^{aB*}	90 ± 5 ^{aB*}	91 ± 5 ^{aB*}	95 ± 4 ^{aA}	< 0.05
Group 3	Heart rate	239 ± 33 ^{bA}	209 ± 18 ^{bB*}	196 ± 17 ^{bC*}	205 ± 19 ^{bB*}	208 ± 29 ^{bB*}	< 0.01
	Respiratory rate	115 ± 18 ^{bA}	58 ± 13 ^{bB*}	56 ± 11 ^{bB*}	61 ± 10 ^{bB*}	69 ± 12 ^{bC*}	< 0.001
	Body temperature	37.3 ± 0.5 ^{bA}	34.3 ± 0.5 ^{bB*}	33.1 ± 0.5 ^{bC*}	32.4 ± 0.5 ^{bD*}	33.5 ± 0.6 ^{bC*}	< 0.001
	SpO ₂	96 ± 3 ^{bA}	88 ± 6 ^{bB*}	86 ± 7 ^{bB*}	88 ± 6 ^{bB*}	93 ± 5 ^{bA*}	< 0.01
Group 4	Heart rate	242 ± 36 ^{cA}	220 ± 17 ^{cB*}	201 ± 16 ^{cC*}	210 ± 18 ^{cB*}	215 ± 24 ^{cB*}	< 0.01
	Respiratory rate	121 ± 16 ^{cA}	54 ± 12 ^{cB*}	52 ± 10 ^{cB*}	56 ± 9 ^{cB*}	63 ± 11 ^{cc*}	< 0.001
	Body temperature	37.4 ± 0.4 ^{cA}	34.1 ± 0.6 ^{cB*}	32.9 ± 0.6 ^{cc*}	32.2 ± 0.6 ^{cD*}	33.2 ± 0.7 ^{cc*}	< 0.001
	SpO ₂	95 ± 4 ^{cA}	86 ± 7 ^{cB*}	85 ± 7 ^{cB*}	87 ± 7 ^{cB*}	92 ± 6 ^{cA*}	< 0.01
Group 5	Heart rate	245 ± 31 ^{dA}	225 ± 16 ^{dB*}	207 ± 15 ^{dc*}	216 ± 17 ^{dB*}	221 ± 22 ^{dB*}	< 0.01
	Respiratory rate	124 ± 15 ^{dA}	50 ± 11 ^{dB*}	49 ± 9 ^{dB*}	53 ± 8 ^{dB*}	59 ± 10 ^{dc*}	< 0.001
	Body temperature	37.5 ± 0.4 ^{dA}	33.9 ± 0.5 ^{dB*}	32.6 ± 0.5 ^{dc*}	31.9 ± 0.5 ^{dD*}	33.0 ± 0.6 ^{dc*}	< 0.001
	SpO ₂	95 ± 4 ^{dA}	84 ± 7 ^{dB*}	83 ± 7 ^{dB*}	85 ± 6 ^{dB*}	91 ± 5 ^{dA*}	< 0.01
Group 6	Heart rate	247 ± 30 ^{eA}	228 ± 15 ^{EB*}	210 ± 14 ^{ec*}	219 ± 16 ^{EB*}	223 ± 20 ^{EB*}	< 0.01
	Respiratory rate	126 ± 14 ^{eA}	48 ± 10 ^{EB*}	47 ± 8 ^{EB*}	51 ± 7 ^{EB*}	57 ± 9 ^{ec*}	< 0.001
	Body temperature	37.4 ± 0.3 ^{eA}	33.7 ± 0.5 ^{EB*}	32.4 ± 0.5 ^{ec*}	31.7 ± 0.4 ^{eD*}	32.8 ± 0.5 ^{ec*}	< 0.001
	SpO ₂	94 ± 5 ^{eA}	83 ± 8 ^{EB*}	82 ± 8 ^{EB*}	84 ± 7 ^{EB*}	90 ± 6 ^{eA*}	< 0.01

Explanations: Different lowercase superscript letters (a-e) within the same column indicate significant intergroup differences ($P < 0.05$). Different uppercase superscript letters (A-D) within the same row indicate significant intragroup temporal differences ($P < 0.05$).

* indicates statistically significant difference compared with baseline (0 min) within the same group.

Group 1: SC medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 2: SC medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)

Group 3: SC medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

Group 4: IP medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 5: IP medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)

Group 6: IP medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

Interestingly, despite receiving identical drug doses, Groups 1 and 4 demonstrated different cardiovascular responses during the early anesthetic period. Group 4 exhibited more rapid induction and earlier heart rate reduction than Group 1. This finding may be explained by the faster systemic absorption associated with intraperitoneal administration. The rapid achievement of effective plasma concentrations following IP administration likely accelerated the onset of medetomidine-induced cardiovascular effects. Similar route-dependent differences have previously been reported in rodents receiving injectable anesthetic agents through different administration routes (6, 20).

The cardiovascular effects observed in the present study cannot be attributed solely to medetomidine. Ketamine itself possesses unique cardiovascular properties that may modify the overall response. Unlike many injectable anesthetic agents, ketamine gener-

ally stimulates the sympathetic nervous system and increases circulating catecholamine concentrations. Consequently, ketamine often increases heart rate and arterial blood pressure when administered alone (15). However, when ketamine is combined with medetomidine, the sympatholytic effects of medetomidine generally predominate. The results of the present study support this concept, as significant reductions in heart rate were observed despite the presence of ketamine in all anesthetic protocols.

The greater cardiovascular depression observed in Groups 4-6 may also reflect the influence of anesthetic depth. Animals anesthetized intraperitoneally achieved surgical anesthesia more rapidly and maintained surgical anesthesia for longer periods. Deeper anesthesia is frequently associated with greater suppression of autonomic nervous system activity and more pronounced cardiovascular depression. Therefore, the lower heart

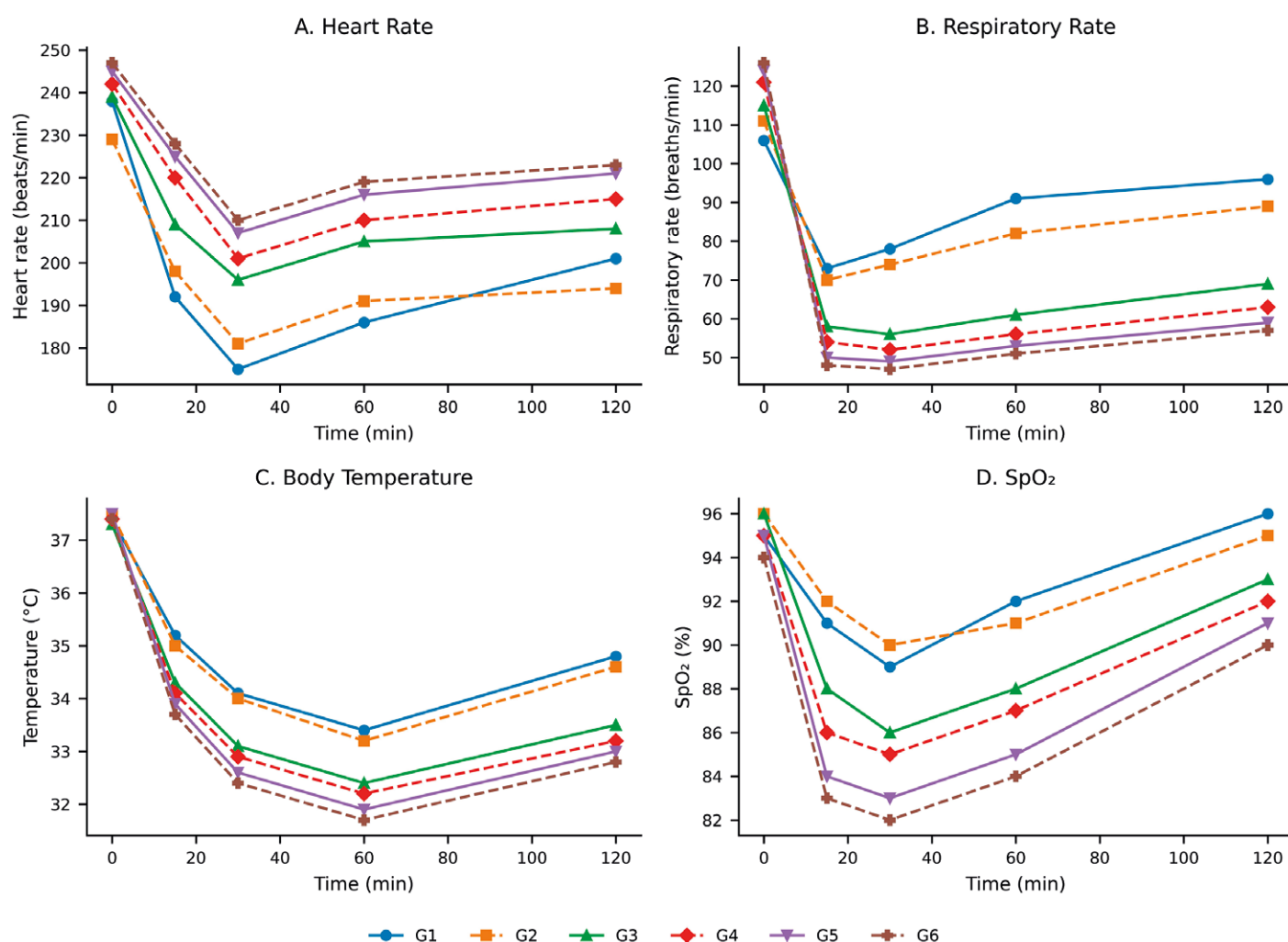


Fig. 1. Temporal changes in cardiopulmonary parameters during anesthesia in rats receiving different ketamine-medetomidine combinations. (A) Heart rate, (B) respiratory rate, (C) body temperature, and (D) peripheral oxygen saturation (SpO₂)

Explanations: Values are presented as mean $\pm$ SD.

Group 1: SC medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 2: SC medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)

Group 3: SC medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

Group 4: IP medetomidine (1 mg/kg) + ketamine (100 mg/kg)

Group 5: IP medetomidine (0.5 mg/kg) + ketamine (75 mg/kg)

Group 6: IP medetomidine (0.5 mg/kg) + ketamine (100 mg/kg)

rate values observed in these groups may result from both pharmacological effects and differences in anesthetic depth.

Although significant bradycardia occurred in all groups, no severe arrhythmias, cardiovascular collapse, or anesthetic-related mortality were observed during the study period. This finding is clinically important because it suggests that the ketamine-medetomidine combinations evaluated in the present study produced predictable cardiovascular depression while maintaining an acceptable safety profile. Similar conclusions have been reported in previous investigations evaluating medetomidine-based anesthesia in laboratory rodents (6, 12).

From a practical perspective, the reductions observed in heart rate emphasize the importance of cardiovascular monitoring during injectable anesthesia. Continuous assessment of heart rate may allow early detection

of excessive cardiovascular depression and facilitate timely supportive interventions. This consideration is particularly relevant in animals receiving higher anesthetic doses or undergoing prolonged surgical procedures. Although the degree of bradycardia observed in the present study remained within clinically manageable limits, the findings indicate that cardiovascular monitoring should be considered an essential component of ketamine-medetomidine anesthesia protocols in laboratory rats. Although no mortality was observed in the present study, previous investigations have demonstrated that anesthetic dose selection remains critical because ketamine-medetomidine anesthesia may be associated with increased anesthetic risk under specific physiological conditions (3).

Respiratory rate. Temporal changes in respiratory rate during anesthesia are presented in Table 2 and Figure 1B. Respiratory rate decreased significantly in

all experimental groups following anesthetic administration ($P < 0.01$). The most pronounced respiratory depression occurred between 15 and 60 min after induction. Group 6 exhibited the greatest reduction, with respiratory rate decreasing from 126 ± 14 breaths/min at baseline to 47 ± 8 breaths/min at 30 min. Similar decreases were observed in Group 5 (124 ± 15 to 49 ± 9 breaths/min) and Group 4 (121 ± 16 to 52 ± 10 breaths/min). Although respiratory rates gradually increased toward the end of the monitoring period, values remained significantly lower than baseline measurements in most groups at 120 min. These findings indicate that ketamine-medetomidine anesthesia induced substantial respiratory depression, particularly in animals receiving intraperitoneal administration and higher anesthetic doses.

Respiratory depression is a well-recognized consequence of α_2 -adrenergic agonist administration. Medetomidine decreases respiratory drive through central nervous system depression and reduced responsiveness of respiratory centers to carbon dioxide. Consequently, respiratory frequency and minute ventilation decline following administration of medetomidine-containing anesthetic protocols (14). Previous investigations in rodents have similarly demonstrated significant reductions in respiratory rate following administration of medetomidine-based anesthetic combinations (14, 17, 18).

The marked respiratory depression observed in Groups 4-6 may reflect both route-dependent and dose-dependent effects. Intraperitoneal administration resulted in more rapid systemic absorption and earlier achievement of effective plasma drug concentrations. Consequently, respiratory centers were exposed more rapidly to pharmacologically active concentrations of medetomidine, resulting in greater suppression of respiratory activity. Furthermore, longer surgical anesthesia duration achieved in these groups may have contributed to the more profound reductions in respiratory rate.

Ketamine causes comparatively less respiratory depression than many other injectable anesthetic agents and is generally considered advantageous because spontaneous respiration is often preserved during anesthesia (13). This characteristic may explain why severe respiratory complications were not observed despite substantial reductions in respiratory rate. Although respiratory frequency decreased significantly in all groups, spontaneous breathing was maintained throughout the study period, and no animal required assisted ventilation. Similar findings have been reported in previous studies evaluating ketamine-containing balanced anesthetic protocols in laboratory rodents (4, 6).

The relationship between respiratory depression and oxygen saturation deserves particular consideration. Reduced respiratory frequency may decrease alveolar ventilation, impair gas exchange, and ultimately

contribute to reductions in arterial oxygenation. The simultaneous decreases observed in respiratory rate and peripheral oxygen saturation in the present study support this interpretation. Similar associations between respiratory depression and oxygen desaturation have previously been reported in rodents anesthetized with α_2 -agonist-containing protocols (14, 18).

From a pharmacological perspective, the interaction between ketamine and medetomidine may explain the respiratory responses observed in this study. Whereas ketamine tends to preserve respiratory function, medetomidine produces dose-dependent respiratory depression. The overall respiratory response therefore reflects the balance between these opposing pharmacological effects. The results of the present study suggest that the respiratory depressant effects of medetomidine become increasingly dominant as anesthetic depth increases, particularly in intraperitoneally treated animals.

Clinically, these findings emphasize the importance of respiratory monitoring during ketamine-medetomidine anesthesia. Continuous assessment of respiratory rate and oxygen saturation may facilitate early detection of respiratory compromise and allow timely intervention. Although no life-threatening respiratory complications occurred during the present study, the substantial reductions observed in respiratory frequency indicate that supplemental oxygen administration should be strongly considered, especially during prolonged procedures and when higher anesthetic doses are employed. Such supportive measures may improve tissue oxygenation, reduce anesthetic risk, and contribute to safer perioperative management of laboratory rats. Comparable respiratory effects have also been described following administration of other injectable anesthetic combinations containing α_2 -adrenergic agonists in rats (1).

Body temperature. Temporal changes in body temperature during anesthesia are presented in Table 2 and Figure 1C. Body temperature decreased progressively in all experimental groups throughout the anesthetic period, and these reductions were statistically significant both within and between groups ($P < 0.001$). The most pronounced hypothermia was observed in Groups 5 and 6. In Group 6, rectal temperature decreased from $37.4 \pm 0.3^\circ\text{C}$ at baseline to $31.7 \pm 0.4^\circ\text{C}$ at 60 min, representing a reduction of approximately 5.7°C . Similar decreases were observed in Group 5 ($37.5 \pm 0.4^\circ\text{C}$ to $31.9 \pm 0.5^\circ\text{C}$) and Group 4 ($37.4 \pm 0.4^\circ\text{C}$ to $32.2 \pm 0.6^\circ\text{C}$). Although partial recovery occurred by the end of the monitoring period, body temperatures remained significantly below baseline values in all groups at 120 min.

Hypothermia is one of the most common complications associated with anesthesia in laboratory rodents and represents a major concern in experimental animal practice. Rats possess a relatively high surface area-

to-body mass ratio, which predisposes them to rapid heat loss during anesthesia. In addition, anesthesia suppresses behavioral and physiological thermoregulatory mechanisms, thereby reducing the animal's ability to maintain normal body temperature (6). Consequently, even relatively short anesthetic procedures may result in clinically significant decreases in core body temperature.

The profound hypothermia observed in the present study is consistent with previous reports evaluating medetomidine-containing anesthetic protocols in rodents. Significant reductions in body temperature have been described following administration of ketamine-medetomidine, medetomidine-midazolambutorphanol, and other α_2 -agonist-based anesthetic combinations (14, 17, 18). Similar findings have also been reported in mice, hamsters, rabbits, and other laboratory species receiving medetomidine-containing balanced anesthesia protocols (10, 17, 18, 20). Collectively, these studies indicate that hypothermia is a predictable consequence of α_2 -adrenergic agonist administration across multiple species.

Several mechanisms may explain the marked decrease in body temperature observed in the present study. Medetomidine induces peripheral vasoconstriction through activation of vascular α_2 -adrenoceptors, altering normal heat distribution and thermoregulatory responses. In addition, central suppression of sympathetic activity reduces metabolic heat production. Decreased muscular activity during anesthesia further contributes to reduced endogenous heat generation. Together, these physiological effects promote progressive heat loss and impaired thermoregulation during anesthesia (14).

The greater hypothermia observed in Groups 4-6 suggests that the route of administration also influenced thermoregulatory responses. Intraperitoneal administration resulted in more rapid anesthetic induction and a longer duration of surgical anesthesia, which may have accelerated the onset of heat loss. Furthermore, animals receiving higher doses of ketamine remained anesthetized for longer periods, thereby extending their exposure to anesthetic-induced impairment of thermoregulation. These factors likely contributed to the significantly lower temperatures observed in the higher-dose and intraperitoneal administration groups.

The relationship between hypothermia and anesthetic duration should also be considered. Progressive decreases in body temperature may alter drug metabolism and delay anesthetic recovery. Reduced body temperature decreases hepatic enzyme activity and slows systemic drug clearance, potentially prolonging anesthetic effects. This mechanism may partially explain the extended anesthesia durations observed in Groups 1, 3, and 4. Similar interactions between hypothermia and prolonged recovery have been reported in previous

laboratory animal anesthesia studies (6, 18). Repeated ketamine-medetomidine anesthesia has likewise been associated with prolonged recovery periods and alterations in physiological responses in rats (9).

From an animal welfare perspective, prevention of anesthesia-associated hypothermia is critically important. Hypothermia may contribute to delayed recovery, cardiovascular instability, impaired immune function, and altered physiological responses. Because experimental outcomes may be influenced by these physiological disturbances, maintenance of normothermia should be considered an essential component of anesthetic management in laboratory rodents. Previous authors have strongly recommended the routine use of external heat support during medetomidine-containing anesthesia protocols (18).

The findings of the present study support these recommendations. The substantial reductions in body temperature observed across all experimental groups indicate that passive environmental warming alone is insufficient to prevent anesthesia-associated hypothermia. Active thermal support, including circulating warm-water blankets, thermostatically controlled heating pads, or forced warm-air systems, should therefore be incorporated into ketamine-medetomidine anesthesia protocols whenever possible. Such measures may improve physiological stability, shorten recovery time, and enhance overall anesthetic safety.

Importantly, despite the marked hypothermia observed in the present study, no mortality or severe anesthetic complications occurred. Nevertheless, the magnitude of temperature reduction indicates that body temperature monitoring should be regarded as a mandatory component of peri-anesthetic monitoring in laboratory rats. Continuous assessment of body temperature may facilitate early intervention and reduce the risk of hypothermia-associated complications during prolonged surgical procedures.

Peripheral oxygen saturation (SpO_2). Temporal changes in peripheral oxygen saturation during anesthesia are presented in Table 2 and Figure 1D. Significant decreases in SpO_2 values were observed in all experimental groups following anesthetic administration ($P < 0.05$). The greatest reductions occurred between 15 and 60 min after induction and were particularly evident in animals receiving intraperitoneal administration. Group 6 exhibited the most pronounced decrease, with SpO_2 values declining from $94 \pm 5\%$ at baseline to $82 \pm 8\%$ at 30 min. Similarly, Group 5 demonstrated a decrease from $95 \pm 4\%$ to $83 \pm 7\%$, whereas Group 4 decreased from $95 \pm 4\%$ to $85 \pm 7\%$ during the same period. Although oxygen saturation gradually improved toward the end of the monitoring period, values remained below baseline levels in several groups at 120 min. These findings indicate that both anesthetic dose and route of administration significantly influenced oxygenation during anesthesia.

The reductions observed in oxygen saturation are likely multifactorial. Respiratory depression induced by medetomidine-containing anesthetic protocols reduces alveolar ventilation and may impair pulmonary gas exchange. Consequently, oxygen uptake decreases and arterial oxygen saturation may decline. The concurrent reductions in respiratory rate and SpO₂ observed in the present study strongly support this mechanism. Similar associations between respiratory depression and oxygen desaturation have previously been reported in rodents anesthetized with α 2-adrenergic agonist-based anesthetic combinations (14, 17, 18).

Medetomidine may also contribute indirectly to reduced oxygenation through its cardiovascular effects. Marked bradycardia and decreased cardiac output may impair tissue perfusion and alter oxygen delivery during anesthesia. Although arterial blood pressure and blood gas analyses were not evaluated in the present study, the simultaneous decreases in heart rate, respiratory rate, and oxygen saturation suggest that cardiopulmonary depression contributed substantially to the reductions observed in SpO₂ values. Similar cardiopulmonary responses have been reported in laboratory animals receiving medetomidine-containing balanced anesthesia protocols (16, 17).

The more pronounced oxygen desaturation observed in Groups 4-6 may be related to both route-dependent and dose-dependent factors. Intraperitoneal administration resulted in faster induction and longer surgical anesthesia duration, which may have enhanced respiratory depression and impaired oxygenation. Furthermore, higher ketamine doses were associated with prolonged anesthesia duration, potentially extending the period during which animals were exposed to reduced pulmonary ventilation. These findings suggest that animals receiving higher-dose and intraperitoneal protocols may be at greater risk of developing clinically relevant hypoxemia.

Previous investigations have reported comparable decreases in oxygen saturation during injectable anesthesia in rodents. Studies evaluating medetomidine-containing anesthetic combinations have demonstrated that oxygen desaturation frequently accompanies reductions in respiratory rate and body temperature, particularly during prolonged anesthesia (14, 18). Similar findings have also been described in rabbits and other small laboratory species receiving α 2-agonist-based anesthetic protocols (10, 20). These observations indicate that oxygen desaturation is a common physiological consequence of balanced injectable anesthesia and should be anticipated during anesthetic management.

An important observation in the present study was that, despite significant decreases in oxygen saturation, no animal developed severe respiratory distress or anesthetic-related mortality. This finding suggests that the ketamine-medetomidine combinations evaluated in this study maintained an acceptable safety

margin under the experimental conditions employed. Nevertheless, SpO₂ values approaching 80% indicate clinically relevant impairment of oxygenation and should not be overlooked. Prolonged exposure to reduced oxygen saturation may adversely affect tissue oxygen delivery and contribute to postoperative physiological disturbances.

From a clinical and experimental perspective, these findings emphasize the importance of oxygen supplementation during injectable anesthesia. Supplemental oxygen administration has been shown to improve arterial oxygenation, reduce the severity of hypoxemia, and increase anesthetic safety in laboratory animals undergoing prolonged procedures (6, 18). The marked reductions in SpO₂ observed in the present study support routine consideration of oxygen supplementation, particularly in animals receiving higher anesthetic doses or intraperitoneal administration.

In addition to oxygen supplementation, continuous pulse oximetry should be regarded as an essential component of anesthetic monitoring. Pulse oximetry provides a non-invasive method for early detection of oxygen desaturation and allows timely intervention before severe hypoxemia develops. When combined with respiratory and cardiovascular monitoring, pulse oximetry may significantly improve anesthetic safety and contribute to refinement of laboratory animal anesthesia protocols.

Overall, the present findings demonstrate that ketamine-medetomidine anesthesia produces significant but generally reversible reductions in oxygen saturation in rats. The greater oxygen desaturation observed in the higher-dose and intraperitoneal administration groups further supports the conclusion that anesthetic dose and administration route are important determinants of cardiopulmonary function during anesthesia. Appropriate monitoring and supportive care are therefore essential to minimize hypoxemia-associated risks and optimize anesthetic outcomes.

Anesthetic reflexes and surgical depth. Evaluation of anesthetic reflexes demonstrated successful induction of surgical anesthesia in all experimental groups. Loss of pedal withdrawal and palpebral reflexes occurred in every animal following administration of the ketamine-medetomidine combinations. Reflex suppression developed significantly more rapidly in the intraperitoneal administration groups than in the subcutaneous administration groups ($P < 0.05$). Furthermore, animals receiving higher anesthetic doses exhibited more prolonged loss of reflexes and maintained deeper surgical anesthesia throughout the observation period. Reflex recovery occurred later in Groups 4, 5, and 6, indicating prolonged anesthetic depth and delayed return of normal neurological responses.

The assessment of anesthetic reflexes remains one of the most practical and widely accepted methods for evaluating anesthetic depth in laboratory rodents. Loss

of pedal withdrawal and palpebral reflexes is generally considered indicative of an adequate surgical plane of anesthesia and is frequently used to determine whether invasive procedures may be performed without causing pain or distress (2, 6). In the present study, the consistent suppression of these reflexes in all animals confirms that the ketamine-medetomidine protocols successfully achieved surgical anesthesia.

The more rapid reflex suppression observed following intraperitoneal administration is consistent with the shorter induction times recorded in these groups. Faster systemic absorption following intraperitoneal injection likely resulted in more rapid penetration of anesthetic agents into the central nervous system, producing earlier onset of unconsciousness and reflex suppression. Similar findings have been reported in previous studies comparing administration routes of injectable anesthetics in rodents (6, 20).

The prolonged loss of reflexes observed in higher-dose groups is likely related to the synergistic pharmacological interaction between ketamine and medetomidine. Ketamine produces dissociative anesthesia primarily through blockade of NMDA receptors, whereas medetomidine induces profound sedation and analgesia through activation of α_2 -adrenoceptors within the central nervous system (15, 16). When administered together, these agents produce a balanced anesthetic state characterized by enhanced analgesia, improved muscle relaxation, and more sustained surgical anesthesia than either agent alone. This synergism likely contributed to the prolonged reflex suppression observed in Groups 1, 3, and 4.

The findings of the present study are consistent with previous reports demonstrating that ketamine-medetomidine combinations provide reliable surgical anesthesia in rats. Comparable loss of pedal withdrawal and palpebral reflexes has been reported in studies evaluating medetomidine-containing anesthetic protocols in laboratory rodents (14, 17). Furthermore, previous investigators have emphasized that α_2 -agonist-based anesthetic combinations improve anesthetic depth and facilitate surgical manipulation while maintaining acceptable safety margins (10, 14).

An important observation was the absence of anesthetic failure in any experimental group. All animals achieved an adequate surgical plane of anesthesia without requiring supplemental anesthetic administration. This finding indicates that both SC and IP administration routes are capable of producing clinically useful anesthesia when appropriate ketamine-medetomidine combinations are employed. However, the differences in induction time, anesthetic duration, and physiological responses suggest that route selection should be based on the requirements of the intended experimental procedure.

From a practical perspective, prolonged anesthetic depth may provide important advantages during

lengthy surgical interventions because repeated anesthetic administration may be avoided. Nevertheless, excessive anesthetic depth may also increase the risk of cardiopulmonary depression and delayed recovery. Therefore, although Groups 1 and 4 produced the most prolonged surgical anesthesia, careful physiological monitoring remains essential when these protocols are used.

Overall, the anesthetic reflex findings confirm that ketamine-medetomidine combinations administered via either subcutaneous or intraperitoneal routes provide reliable and effective surgical anesthesia in rats. The more rapid onset observed following intraperitoneal administration and the prolonged anesthetic depth associated with higher-dose protocols may be advantageous for specific experimental applications, provided that appropriate monitoring and supportive care are maintained.

The present study provides important information regarding the practical use of ketamine-medetomidine combinations in laboratory rats. The findings demonstrate that both anesthetic dose and route of administration significantly influence induction characteristics, anesthetic duration, anesthetic depth, and cardiopulmonary responses. Intraperitoneal administration consistently produced more rapid induction and deeper anesthesia, whereas higher-dose protocols resulted in prolonged anesthesia accompanied by greater physiological depression. These observations may assist researchers and laboratory animal veterinarians in selecting anesthetic protocols according to the duration and requirements of specific experimental procedures.

From a clinical perspective, Groups 1 and 4 may be particularly suitable for procedures requiring prolonged surgical anesthesia because they provided the longest surgical anesthetic periods. In contrast, lower-dose protocols may be preferable when shorter procedures and more rapid recovery are desired. Regardless of the protocol selected, the significant reductions observed in heart rate, respiratory rate, body temperature, and oxygen saturation emphasize the necessity of continuous physiological monitoring throughout anesthesia.

The present findings are consistent with previous and recent experimental studies demonstrating that ketamine-medetomidine anesthesia provides reliable surgical anesthesia in rats while producing dose-dependent alterations in cardiopulmonary variables, including heart rate, respiratory rate, and oxygen saturation. Recent investigations have also highlighted that repeated administration of ketamine-medetomidine may increase anesthetic risk and mortality, emphasizing the importance of careful physiological monitoring and appropriate perioperative supportive care during experimental procedures. Our findings further extend the current knowledge by directly comparing different ketamine-medetomidine dose combinations administered via both subcutaneous and intraperitoneal routes

under standardized experimental conditions, thereby providing practical information for selecting anesthetic protocols according to the objectives and expected duration of laboratory procedures (23-25).

The study provides a comprehensive comparison of multiple ketamine-medetomidine dose combinations administered through two commonly used routes in laboratory rats. The absence of mortality or severe anesthetic complications, together with the consistent achievement of surgical anesthesia, supports the practical applicability of these protocols. Future studies incorporating invasive cardiovascular monitoring, blood gas analysis, and postoperative recovery assessments may further refine injectable anesthesia protocols and contribute to improved animal welfare and experimental reliability.

This study has several limitations that should be acknowledged. First, arterial blood pressure and blood gas analyses were not performed, which would have provided more detailed information regarding cardiovascular and respiratory function during anesthesia. Second, recovery quality was assessed clinically, whereas objective behavioral scoring systems were not employed. Finally, only healthy adult rats were included in the present study; therefore, the results should be interpreted cautiously when extrapolating to diseased or aged animals. Future studies incorporating invasive hemodynamic monitoring, blood gas analysis, and standardized recovery scoring would further improve the understanding of ketamine-medetomidine anesthesia in laboratory rodents.

The present findings indicate that both the dose of ketamine-medetomidine and the route of administration substantially influence anesthetic quality and physiological responses in rats. Rather than demonstrating a universal superiority of one protocol, our results suggest that the optimal anesthetic regimen should be selected according to the objectives and expected duration of the experimental procedure. These observations may assist investigators in balancing anesthetic depth, duration, and cardiopulmonary safety while maintaining animal welfare.

In conclusion, ketamine-medetomidine combinations administered via both subcutaneous and intraperitoneal routes produced effective surgical anesthesia in rats. However, the anesthetic characteristics and cardiopulmonary responses varied according to both dose and administration route. Selection of the most appropriate protocol should therefore be based on the objectives and anticipated duration of the experimental procedure. Additional studies evaluating blood pressure, blood gas variables, recovery quality, and perioperative supportive interventions are warranted.

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