Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Biochemical Effects of Anesthetic Agents in Laboratory Rats: A Narrative Review

Bougherara Hithem,* Bouchoukh Aya Sofia and Boukhechem Saïd

Gestion de la Santé et Productions Animales ResearchLaboratory, Institut des Sciences Vétérinaires El-Khroub, Université de Constantine 1 Frères Mentouri, Constantine 25000, Algeria

Abstract

ANESTHESIA is an indispensable component of research using laboratory rats (*Rattus norvegicus*), facilitating complex procedures while ensuring animal welfare. However, anesthetic agents are potent pharmacological compounds that can induce significant alterations in an animal's biochemical milieu, challenging data integrity. This narrative review synthesizes and critically evaluatesthe literature on the effects of common anesthetic protocols on key biochemical parameters in rats. Our analysis reveals distinct biochemical signatures for different anesthetic classes. Regarding glucose homeostasis, α2-adrenergic agonists like xylazine induce profound hyperglycemia by suppressing insulin secretion. Dissociative anesthetics such as ketamine also elevate glucose, rendering protocols containing them potentially may confound metabolic endpointswithout careful consideration and control. In renal function, a common finding is a transient elevation in blood urea nitrogen (BUN), primarily due to hemodynamic artifacts like anesthetic-induced hypotension, rather than direct nephrotoxicity. The impact on hepatic enzymes is variable, yet modern inhalants such as isoflurane and sevoflurane exhibit a superior safety profile due to minimal hepatic metabolism. Finally, for neuroendocrine studies, ketamine is a potent activator of the hypothalamic-pituitary-adrenal (HPA) axis, confounding stress-related research by significantly elevating corticosterone. This review concludes that anesthetic selection is a critical methodological decision. It is essential to select a protocol based on study-specific endpoints, incorporate appropriately anesthetized control groups, and transparently report all protocol details to ensure the validity and reproducibility of preclinical findings.

Keywords: Anesthesia, Laboratory Rat, Biochemical Parameters, Ketamine, Xylazine.

Introduction

The laboratory rat (*Rattus norvegicus*) is a cornerstone of modern biomedical science. Its physiological and genetic tractability has made it an essential model for advancing our understanding of human disease, toxicology, and pharmacology [1, 2]. Central to the ethical and scientific conduct of research with these animals is the use of anesthesia. It is an absolute requirement for any procedure that may cause more than momentary pain or distress, ensure animal welfare while enabling the precision required for complex experimental manipulations [3, 4]. Proper perioperative care and selection of anesthesia are critical for both animal welfare and data quality [5].

However, this necessary practice introduces a significant methodological complication: the anesthetic agent itself becomes a variable with the potential to influence experimental outcomes profoundly. Anesthetic drugs, by their very nature, induce a cascade of physiological changes that extend far beyond the central nervous system, altering cardiovascular dynamics, respiratory patterns, and metabolic pathways [6, 7]. These changes can directly or indirectly modify the biochemical parameters a researcher aims to measure, creating a significant risk of data confounding [8]. An observed change in a biomarker—be it glucose, a liver enzyme, or a renal marker—could be incorrectly attributed to the experimental treatment when it is, in fact, a predictable artifact of the chosen anesthetic protocol. This risk undermines the internal validity of a study and can lead to flawed conclusions, wasted resources, and the unnecessary use of animals.

The challenge is amplified by the sheer variety of available anesthetic agents and protocols, each with a unique pharmacological profile. Choices range from injectable agents like ketamine, often combined with

*Corresponding authors: Bougherara Hithem1, E-mail: hithem.bougherara@umc.edu.dz, Tel.: 00213697798902
(Received 12 August 2025, accepted 08 October 2025)
DOI: 10.21608/ejvs.2025.413465.3039

xylazine, to inhalants such as isoflurane and sevoflurane [9, 10]. While the literature contains numerous studies documenting the biochemical impact of these agents, the findings are often scattered across different disciplines and experimental contexts. This narrative review aims to synthesize this body of knowledge, focusing on the problematic nature of anesthetic interference with key biochemical parameters in the laboratory rat, and to provide a critical evaluation of the underlying mechanisms and their implications for experimental design.

Literature Search Strategy

This narrative review was compiled through a targeted search of scientific literature. We utilized the PubMed, Scopus, and Google Scholar databases for articles published up to 2024. The search strategy involved a combination of keywords, including "anesthesia", "laboratory rat", "*Rattus norvegicus*", "biochemical parameters", "ketamine", "xylazine", "isoflurane", "sevoflurane", "alfaxalone", "glucose", "hyperglycemia", "blood urea nitrogen", "creatinine", "ALT", "AST", and "corticosterone". Inclusion criteria were: (1) original research articles, reviews, and veterinary textbooks; (2) studies conducted on laboratory rats; (3) articles published in English. Studies on other species were consulted for mechanistic insights but are specified as such in the text.

Anesthetic-Induced Alterations of Key Biochemical Systems

Overview of Common Anesthetic Agents

The anesthetic agents discussed fall into several main categories. **Injectable anesthetics** include dissociative agents like ketamine (an NMDA receptor antagonist) and α2-adrenergic agonists like xylazine (providing sedation and analgesia), which are often used in combination [10]. Newer injectable agents include neurosteroids like alfaxalone [11]. **Inhalant anesthetics**, such as isoflurane and sevoflurane, are volatile liquids valued for their rapid onset, recovery, and precise control over anesthetic depth.

Disruption of Glucose Homeostasis

Anesthetic-induced hyperglycemia is a frequently reported and problematic interference, particularly for metabolic and endocrine research [12].

Alpha-2 Adrenergic Agonists: Xylazine is a potent inducer of hyperglycemia [13, 14]. The mechanism involves stimulation of α2-adrenoceptors on pancreatic β-cells, which directly suppresses insulin secretion [15]. Simultaneously, it promotes glucagon release and stimulates hepatic glycogenolysis [6, 16], making protocols containing it, such as the ketamine-xylazine cocktail, unsuitable for studies where glucose metabolism is a primary endpoint [17]. Recent studies continue to highlight these significant hyperglycemic effects compared to other regimens [18].

Ketamine: As a dissociative anesthetic, ketamine also contributes to hyperglycemia [19, 20], primarily through sympathetic nervous system stimulation and subsequent catecholamine release. The magnitude can be modulated by co-administered drugs like acepromazine, which may blunt this response [19].

*Newer Agents and Inhalants:*Alfaxalone has demonstrated a limited impact on blood glucose, offering a more stable alternative [11, 20]. This is supported by recent comparative studies, which found that alfaxalone maintained more stable glucose and lactate levels compared to the significant hyperglycemia induced by ketamine-xylazine during surgical procedures in rats [21]. Inhalant anesthetics like isoflurane and sevoflurane may cause mild hyperglycemia, likely linked to the surgical stress response rather than a direct pharmacological effect [22, 23].

Alterations in Markers of Renal Function

Blood urea nitrogen (BUN) and creatinine are standard biomarkers for renal health [24]. Anesthetics influence these primarily through hemodynamic effects.

Pre-renal Azotemia: A frequent finding is a transient elevation in BUN post-anesthesia [25]. This is typically not direct nephrotoxicity but pre-renal azotemia. Many anesthetics cause dose-dependent vasodilation and hypotension [3, 26], leading to a temporary reduction in renal blood flow (RBF) and glomerular filtration rate (GFR) [27]. As urea reabsorption is inversely related to flow rate, reduced RBF increases its serum concentration [24].

Creatinine Stability: In contrast, creatinine clearance is less dependent on tubular flow, so its levels often remain stable during these shifts [25, 28]. Supplemental intravenous fluids during anesthesia are critical to mitigate these effects [4]. Recent comparisons in rats confirm that inhalants like sevoflurane tend to preserve renal biomarkers more effectively than some injectable combinations [29]. Further supporting this, a recent head-to-head comparison demonstrated that sevoflurane resulted in less pronounced changes in renal injury biomarkers compared to isoflurane in rats, suggesting it may offer superior renal protection [30].

Impact on Hepatic Enzyme Activity

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are measured to assess hepatocellular integrity. The literature presents a complex picture, posing a challenge for toxicological studies [25, 31]. Propofol is generally considered safe, though prolonged infusions can be a metabolic load [32].

Inhalant Anesthetics: Halothane, now rarely used, was known to cause "halothane hepatitis" [33]. Modern inhalants like isoflurane and sevoflurane have a much higher safety profile due to minimal hepatic metabolism (<1% for isoflurane and ~2-5% for sevoflurane) [34, 35]. However, minor, transient increases in liver enzymes can occur after prolonged exposure, possibly from altered hepatic blood flow or mild hypoxic stress [36]. Recent studies in rats found sevoflurane to have minimal effect on hepatic biomarkers [29].

Effects on the Hypothalamic-Pituitary-Adrenal (HPA) Axis

The stress of handling and injection, and the anesthetic's action, can alter corticosterone, the primary stress hormone in rats.

Ketamine: Ketamine is a potent activator of the HPA axis, leading to a significant and rapid increase in plasma corticosterone [37]. This makes it a critical confounder for behavioral, neuroendocrine, and immunological research.

Inhalant Anesthesia: In contrast, isoflurane or sevoflurane may result in a less pronounced corticosterone response, especially after the initial induction period [38].

Alfaxalone and Sevoflurane: Newer agents like alfaxalone have also been shown to activate the HPA axis [39]. Anesthesia with sevoflurane in neonatal rats has also been shown to cause an acute stress response [40]. Similarly, recent research has confirmed that even short-term sevoflurane exposure can elicit a significant, albeit transient, corticosterone stress response, underscoring the need for careful consideration in neuroendocrine studies [41].

Discussion

Navigating an Inescapable Methodological Challenge

The evidence confirms that anesthesia is an active pharmacological intervention. These consequences can mimic, mask, or modify the biological signals researchers intend to study, making the choice of anesthesia a critical aspect of experimental design. The mechanisms are primarily threefold:

Direct Pharmacological Action (e.g., xylazine's inhibition of insulin release).

Indirect Physiological Responses (e.g., ketamine-induced catecholamine release).

Systemic Hemodynamic Compromise (e.g., hypotension affecting organ function).

This understanding has profound implications. A study investigating glucose metabolism using xylazine without appropriate controls is fundamentally flawed. A toxicology study must account for the anesthetic's baseline effect on hepatic enzymes. The principle of "balanced anesthesia," using multiple drugs at lower doses to mitigate side effects, is a valuable strategy [9]. Furthermore, an appropriately anesthetized control group is essential to isolate the treatment effect from the anesthetic's "noise."

To aid researchers in this critical decision-making process, a decision tree is presented in Figure 1. This practical tool guides the selection of an anesthetic protocol based on the primary focus of the study. The process begins by identifying the main experimental area (e.g., Metabolic, Renal, Hepatic, or Neuroendocrine). Following the corresponding branch leads to specific recommendations, such as which agents to avoid (e.g., xylazine for metabolic studies) and which to consider (e.g., inhalants for hepatic studies). The flowchart converges on universal principles that are paramount for all research: the mandatory use of a dedicated, anesthetized control group and the thorough, transparent reporting of all protocol details [5, 42].

Limitations and Future Directions

This narrative review has limitations. The search was not systematic, which may introduce selection bias. Furthermore, heterogeneity in study designs, dosages, and rat strains makes direct quantitative comparisons challenging. Future research should focus on direct, head-to-head comparisons of newer agents like alfaxalone against modern inhalants under standardized conditions. Mechanistic studies exploring the molecular pathways behind anesthetic-induced organ stress are warranted. Finally, more research is needed on the long-term effects of repeated anesthesia in chronic study models.

Conclusion and Recommendations

The confounding influence of anesthetics on biochemical parameters is a significant challenge. No single protocol is universally optimal. The selection must be a deliberate, evidence-based process. We propose the following recommendations:

Prioritize Endpoint Stability: The primary consideration must be the anesthetic's potential to interfere with the study's key endpoints. Avoid protocols known to induce hyperglycemia (e.g., ketamine-xylazine) for metabolic research.

Understand the Mechanism of Interference: Recognizing that a rise in BUN is likely due to a temporary hemodynamic shift allows for more accurate data interpretation.

Refine and Standardize Protocols: Employ balanced anesthesia where appropriate. Standardize all related procedures (fasting, time of day, duration of anesthesia) to minimize variability.

Embrace Transparent Reporting: Publications must include a detailed description of the anesthetic

protocol (all drugs, doses in mg/kg, route of administration, and duration) to ensure replication.

By treating the choice of anesthesia with scientific rigor, researchers can enhance the quality and translational value of their work. This approach indirectly supports Sustainable Development Goal 3 (Good Health and Well-being) by enhancing the reliability of preclinical findings.

Acknowledgments

The authors would like to express their sincere gratitude to all members of the "Gestion de la Santé et Productions Animales" Research Laboratory at the Institut des Sciences Vétérinaires El-Khroub, Université Constantine 1, for their valuable support and stimulating scientific discussions that contributed to the development of this review. Special thanks to the technical staff for their assistance throughout the research process.

Funding Statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval:

Not applicable.

TABLE 1. Summary of Key Biochemical Effects of Common Anesthetic Protocols in Laboratory Rats. This table provides a quick-reference comparison of different anesthetic agents, highlighting their typical dose ranges and their documented effects on glucose, renal biomarkers, hepatic enzymes, and stress hormones to aid in protocol selection.

Anesthetic Protocol	Typical Dose Range in Rats	Glucose (Insulin) Effects	Renal Biomarkers (BUN, Creatinine)	Hepatic Enzymes (ALT, AST)	Stress Hormones (Corticosterone)
Ketamine-Xylazine	K: 75-100 mg/kg; X: 5-10 mg/kg (IP)	**Significant hyperglycemia** due to α2 agonist inhibition of insulin secretion [13-17].	**Transient BUN elevation** from hypotension-induced reduced renal perfusion [3, 24-27].	Variable; mild transaminase elevations reported [25, 31, 42].	**High**; potent activator of HPA axis, significantly increases corticosterone [37].
Alfaxalone	10-15 mg/kg (IV) or 20-30 mg/kg (IP)	**Minimal effect** on glucose homeostasis; a preferred agent for metabolic studies [11, 20, 21].	**Minimal impact**; tends to preserve renal perfusion [11].	**Minimal hepatotoxicity** reported [11].	Shows a **notable increase** in corticosterone, indicating HPA axis activation [39].
Isoflurane	Induction: 4-5%; Maintenance: 1.5-2.5%	**Mild to moderate hyperglycemia**, likely via stress-induced catecholamine release [22, 23].	**Minimal BUN changes**; hemodynamic stability is generally better than injectables [29, 35].	**Minimal changes**; <1% is metabolized in the liver, high safety profile [34-36].	**Moderate**; less HPA activation than injectable protocols, especially after induction [38].
Sevoflurane	Induction: 5-7%; Maintenance: 2.5-3.5%	**Comparable or slightly less glycemic effect** than isoflurane; offers good stability [22, 23].	**Slightly better renal biomarker stability** than isoflurane; preserves renal blood flow well [29, 30, 35].	**Minimal hepatotoxicity**; ~2-5% metabolism, faster elimination [29, 34-36].	Causes an acute stress response on induction; a **controllable option** for neuroendocrine studies [40, 41].

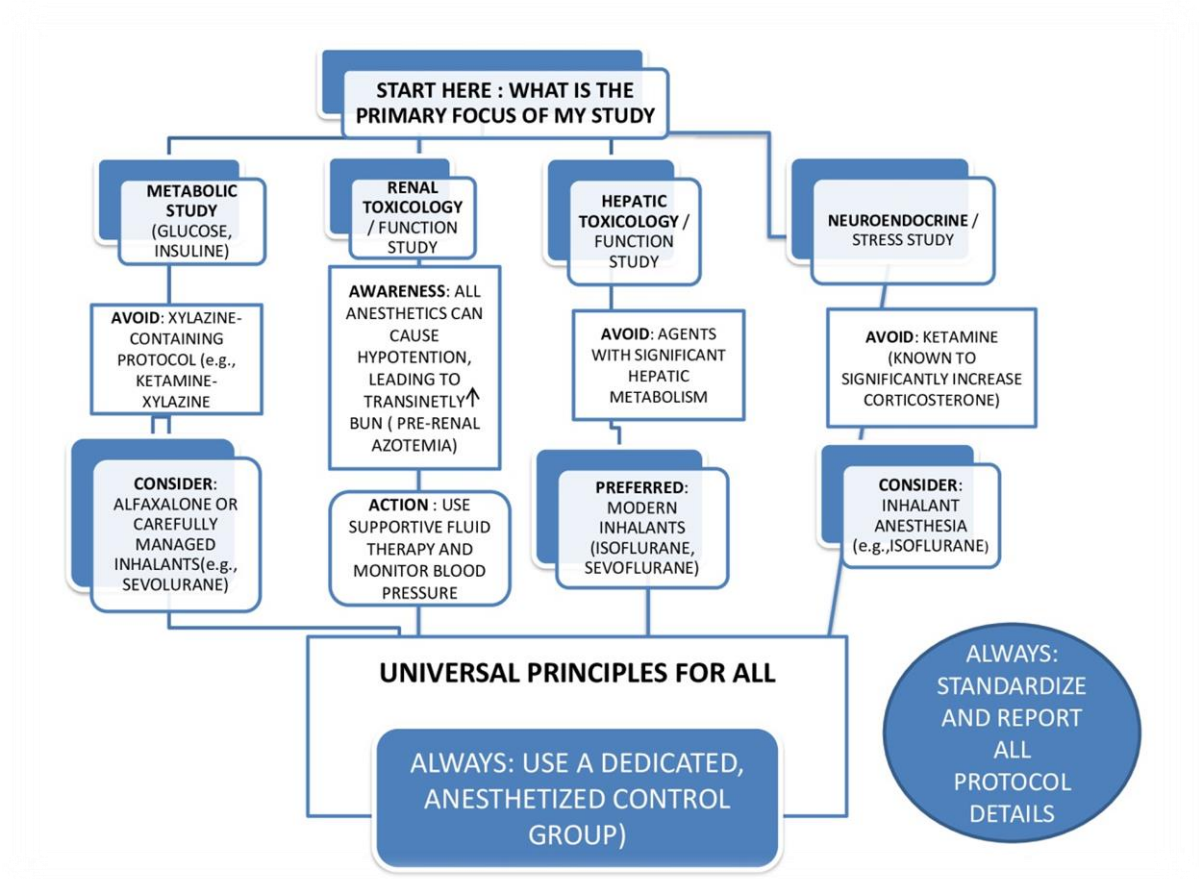

Fig. 1. A Decision-Making Framework for Selecting an Anesthetic Protocol in Rat-Based Research.
This flowchart guides researchers from their primary study focus (e.g., metabolic, renal) to recommended anesthetic agents to consider or avoid, culminating in universal best practices applicable to all experimental designs.

References

1. Hrapkiewicz, K., Medina, L. and Holmes, D.D. Clinical Medicine of Small Mammals and Primates, 2nd ed.,Teton New Media,(1998).
2. Hedrich, H.J. The Laboratory Rat. *Handbook of Experimental Animals*. Academic Press,(2004).
3. Flecknell, P. and Waterman-Pearson, A. Pain Management in Animals. W.B. Saunders, (2000).
4. Grimm, K.A., Lamont, L.A., Tranquilli, W.J., Greene, S.A. and Robertson, S.A.Veterinary Anesthesia and Analgesia. 5th ed. Ames, IA, Wiley-Blackwell, (2015).
5. Stenberg, M. Anaesthesia and Perioperative Care for Rodents. In: *Methods in Molecular Biology*. Vol. 2412. Humana, New York, NY,(2022).
6. Muir, W.W., Hubbell, J., Skarda, R.L. and Bednarski, R.M. Handbook of veterinary anesthesia. 2nd ed., St. Louis, Mosby,(1995).
7. Thurmon, J.C. and Short, C.E. History and overview of veterinary anesthesia. In: Tranquilli, W.J., Thurmon, J.C. and Grimm, K.A.(editors), *Lumb and Jones' Veterinary Anesthesia and Analgesia*. 4th ed. Blackwell Publishing, Ames, 2007,pp.3-9.
8. Carregaro, A.B., Gehrcke, M.I., Tironi, S.M.P. and da Cruz, F.S.F. Effects of different anesthetic protocols on the clinical parameters of Wistar rats. *Ciencia. Rural,* **37**(4), 1047-1052 (2007).
9. Thiebault, J.J. Les agents de la pré-anesthésie chez les carnivores domestiques. *Point. Vét.*, **25**(153), 27-33 (1993).
10. Pacharinsak, C. and Smith, J. Anesthetic and analgesic considerations for the laboratory rat. *J. Am. Assoc. Lab. Anim. Sci.*, **60**(2), 143-156 (2021).
11. Chen, Y., Zhao, M., Liu, Y., Li, H., Luo, H. and Zhang, Y. Effects of alfaxalone, propofol, and ketamine-xylazine on biochemical and hematological variables in New Zealand white rabbits. *J. Am. Assoc. Lab. Anim. Sci.*, **59**(4), 428-435 (2020).

12. Hsu, W.H. and Hembrough, F.B. Intravenous glucose tolerance test in cats: influenced by acetylpromazine, ketamine, morphine, thiopental, and xylazine. *Am. J. Vet. Res.*, **43**(11), 2060-2061(1982).

13. Ambrisko, T.D. and Hikasa, Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. *Can. J. Vet. Res*., **66**(1), 42-49 (2002).

14. Robertson RP, Porte D Jr, Bierman EL. Adrenergic modulation of basal insulin secretion in man. *Diabetes*, **22**(1),1-8(1973). doi:10.2337/diab.22.1.1

15. Botana, L.M., Landoni, F. and Martin-Jimenez, T. Pharmacokinetics of a xylazine-ketamine combination in dogs. *J. Vet. Pharmacol. Ther*., **19**(4), 291-296 (1996).

16. Saha, S., Batabyal, S., Bera, A. and Jana, S. Effects of ketamine, xylazine and their combination on blood glucose and serum insulin levels in goats. *Vet. World*, **6**(8), 541-544 (2013).

17. Verstegen, J., Fargetton, X., Donnay, I. and Ectors, F. An evaluation of the clinical utility of a new medetomidine-ketamine-buprenorphine combination for surgical procedures in rats. *Lab. Anim*., **25**(1), 16-21 (1991).

18. Kumar, N., Kumar, A. and Singh, B. Haematological and biochemical effects of ketamine with and without meperidine and diazepam in dogs. *Ind. J. Anim. Sci*., **59**(7), 815-818 (1989).

19. Thurmon, J.C., Tranquilli, W.J. and Benson, G.J. Preanesthetics and anesthetic adjuncts. In: *Lumb and Jones' Veterinary Anesthesia*. 3rd ed. Philadelphia: Lea &Febiger; 1997, p. 183-203.

20. Pascoe, P.J., Kita, K., Pypendop, B., Lindsley, S. and Siao, K. The effect of alfaxalone on blood glucose concentrations in cats. *Vet. Anaesth. Analg*., **48**(1), 36-42 (2021).

21. García-T-de-Alba, C., Canfrán, S., Cediel, R. and Gómez-de-Segura, I.A. Metabolic and cardiorespiratory effects of alfaxalone or ketamine-xylazine anesthesia for ovariohysterectomy in rats. *Animals*, **12**(15), 1949 (2022).

22. Gargiulo, S., Greco, A., Gramanzini, M., Esposito, S., Ciotola, F., D'Angelo, D. and Iannotti, L. A comparison of the effects of sevoflurane and isofluraneanaesthesia on the B-cell function in a rat model. *BMC Veterinary Research*, **14**(1), 294 (2018).

23. Redfors, B., Sjöquist, P.O., Jansson, P.A. and Ricksten, S.E. Sevoflurane and isoflurane cause similar stress responses but only sevoflurane causes hyperglycemia and hyperinsulinemia in a porcine model of surgical stress. *Anesthesia & Analgesia*, **110**(3), 795–802 (2010).

24. Guyton, A.C. and Hall, J.E. Guyton and Hall Textbook of Medical Physiology. 13th ed. Elsevier,(2016).

25. Gonzalez-Gil, A., Illera, J.C., Silvan, G. andIllera, M. Effects of the anaesthetic/tranquillizer treatments on selected plasma biochemical parameters in NZW rabbits. *Lab. Anim*., **37**(2), 155-161 (2003).

26. Ilkiw, J.E., Pascoe, P.J., Haskins, S.C. and Patz, J.D. Cardiovascular and respiratory effects of propofol administration in hypovolemic dogs. *Am. J. Vet. Res*., **53**(11), 2323-2327 (1992).

27. Kästner, S.B.R., Kietzmann, M., Clemens, S. andKuczka, A. Effects of propofol and medetomidine on systemic haemodynamics and regional blood flow in beagle dogs. *Vet. Anaes. Analg*., **33**(2), 83-93 (2006).

28. Finco, D.R. Evaluation of renal functions. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L., editors. *Clinical biochemistry of domestic animals*. 6th ed. Academic Press, p. 461-494 (2008).

29. Lee, J.C., Park, Y.H., Lee, J.H., Kim, Y.B., Choi, J.H., Lee, Y.K., Kim, D.J., Lee, K.S., Cheong, J.H. and Kang, B.Y. Comparison of the effects of isoflurane and sevoflurane on renal and hepatic functions in rats. *Inhalation Toxicology*, **24**(11), 739–746 (2012).

30. Pereira, S., Valente, P., Antunes, L., Guedes de Pinho, P. and Carvalho, F. The effects of sevoflurane versus isoflurane on renal biomarkers during elective surgery in rats. *Veterinary Anaesthesia and Analgesia*, **50**(3), 250–258 (2023).

31. Valentim, A.M., Guedes, A.G., Taga, A. and Cortopassi, S.R. Effects of isoflurane and sevoflurane on variables of physiologic importance in Wistar rats. *J. Am. Assoc. Lab. Anim. Sci*., **54**(2), 162-168 (2015).

32. ZuranPonjevic, M., Zivkovic-Radivojevic, V., Gvozdic, D., Vucinic, M., Stepanovic, P. and Radisavljevic, K. Effects of propofolanaesthesia on haematological and biochemical parameters in dogs. *Acta VeterinariaHungarica*, **62**(4), 442–453 (2014).

33. Steffey, E.P. Inhalation anesthetics. In: Thurmon, J.C., Tranquilli, W.J., Benson, G.J., editors. *Lumb and Jones' Veterinary Anesthesia*. 3rd ed. Philadelphia: Lea &Febiger, p. 297-323 (1997).

34. Eger, E.I.,Koblin, D.D., Bowland, T., Ionescu, P., Laster, M.J., Fang, Z., Gong, D., Sonner, J. And Weiskopf, R.B. The minimum alveolar anestheticconcentrationofsevoflurane in humans. *Anesthesia&Analgesia*, **84**(2), 398–403 (1997).

35. Cretcher, S., R-Richardson and C., Anwer, M. A comparison of sevoflurane and isoflurane on recovery and selected biochemical parameters in the Wistar rat. *Contempo. Top Lab. Anim. Sci*., **43**(5), 12-15 (2004).

36. Jaber, S.M., Hankenson, F.C., Heng, K., McKinstry-Wu, A., Kelz, M.B. and Marx, J.O. Anesthetic and physiologic effects of isoflurane, sevoflurane, and their combination in laboratory mice. *J. Am. Assoc. Lab. Anim. Sci*., **56**(6), 734-743 (2017).

37. Vicentic, A., O'Malley, M., O'Reilly, M., Lee, B.S., O'Dwyer, A.M., Jones, S.R., Krystal, J.H. and D'Souza, D.C. The effects of repeated ketamine administration on the HPA axis and its response to a subsequent acute stress. *Psychoneuroendocrinology*, **31**(4), 511–523 (2006).

38. Gonzalez-Gil, A., Silvan, G. and Illera, J.C. Pituitary-adrenocortical axis, serum serotonin and biochemical response after halothane or isoflurane anaesthesia in rabbits. *Lab. Anim*., **41**(4), 411-419 (2007).

39. Ciğremci, B. and Binici, C. Comparison of the Effects of Ketamine-Midazolam and Alfaxalone-Midazolam Sedation on Cardiopulmonary, Haematological and Biochemical Values in New Zealand Rabbits. *Kocatepe Vet. J*., **13**(1), 101-109 (2020).

40. Vutskits, L. and Gascon, E. Anesthesia with sevoflurane in neonatal rats: developmental neuroendocrine abnormalities and alleviating effects of the corticosteroid and Cl− importer antagonists. *J. Neurosci*., **35**(28), 10300-12 (2015).

41. Lee, S., Kim, D., Kim, H., Han, H. and Han, J.S. Effects of short-term sevofluraneanesthesia on stress responses in Sprague-Dawley rats. *Laboratory Animal Research*, **39**(1), 12 (2023).

42. Farver, T.B., Haskins, S.C. and Patz, J.D. Cardiopulmonary effects of acepromazine and subsequent administration of ketamine in dog. *Am. J. Vet. Res*., **47**(3), 631-635 (1986).