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Effects of intraperitoneal tramadol and flunixin on pain and stress indices following ovariohysterectomy in rabbits

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Abstract

Background: Effective postoperative pain management is essential for improving recovery and promoting animal welfare in veterinary surgery. Rabbits are highly sensitive to surgical stress, and limited information is available regarding the comparative efficacy of intraperitoneally administered analgesics following ovariohysterectomy.

Methods: Fifteen healthy adult female New Zealand White rabbits weighing 1900-2500 g were randomly allocated into three groups (n = 5/group): control, tramadol (5 mg/kg, intraperitoneal), and flunixin meglumine (1 mg/kg, intraperitoneal). Animals were housed individually under controlled environmental conditions after a one-week acclimatization period. Following ovariohysterectomy, heart rate, respiratory rate, and rectal temperature were recorded at 0, 2, and 6 h postoperatively. Postoperative pain was assessed using the Rabbit Grimace Scale at 0, 1, 3, 6, 12, and 24 h. Serum cortisol and melatonin concentrations were measured using rabbit-specific ELISA kits, and serum glucose was analyzed using an automated biochemical analyzer. Data were analyzed using SPSS version 26. The level of statistical significance was set at 0.05.

Results: Postoperative stress was most pronounced during the second hour after surgery and was characterized by increased heart rate, respiratory rate, cortisol, and glucose concentrations, along with decreased melatonin levels. Both tramadol and flunixin significantly reduced physiological pain indices, such as heart and respiratory rates, compared with the control group (P-Value<0.001), while behavioral pain indices also decreased following treatment. Tramadol was more effective in attenuating early physiological stress responses, whereas flunixin provided more sustained control of behavioral pain during later postoperative time points. However, differences in some biochemical variables among treatment groups were not statistically significant.

Conclusion: Intraperitoneal administration of tramadol and flunixin reduced postoperative pain- and stress-related responses following ovariohysterectomy in rabbits. Tramadol showed greater efficacy during the early postoperative phase, whereas flunixin provided longer-lasting behavioral analgesia. These findings suggest that both agents may be useful for postoperative pain management in rabbits, although further studies with larger sample sizes are required.

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Highlights

What is current knowledge?

- Ovariohysterectomy induces significant postoperative pain and physiological stress responses in rabbits.
- Tramadol and flunixin are commonly used analgesics for perioperative pain management in veterinary medicine.
- The Rabbit Grimace Scale is a validated tool for assessing pain intensity in laboratory rabbits.

What is new here?

- This study directly compares the intraperitoneal analgesic effects of tramadol and flunixin after rabbit ovariohysterectomy.
- Flunixin provided more sustained postoperative analgesia, whereas tramadol more effectively reduced early stress responses.
- Changes in cortisol, glucose, and melatonin were simultaneously evaluated as integrated biomarkers of postoperative stress and pain.

Introduction

Postoperative pain remains a paramount clinical concern in both human and veterinary medicine. Adequate pain management is essential not only for ethical considerations and animal welfare but also for mitigating postoperative complications, accelerating recovery, and minimizing the physiological and metabolic disturbances associated with surgical stress (1,2). Poorly managed acute postoperative pain can lead to delayed recovery and, in certain instances, the transition to chronic pain states (3,4).

Rabbits (Oryctolagus cuniculus) are widely used in veterinary practice and biomedical research; however, pain assessment in this species is particularly challenging because rabbits inherently mask overt signs of discomfort. Consequently, accurate evaluation of postoperative pain in rabbits requires an integrated approach combining behavioral, physiological, and biochemical indicators (5,6). Among behavioral assessment tools, the Rabbit Grimace Scale has emerged as a reliable, validated method for quantifying pain intensity through subtle changes in facial expression (7,8).

Ovariohysterectomy is a routine soft-tissue surgical procedure in both clinical veterinary practice and experimental studies. Because it involves an abdominal wall incision and manipulation of the visceral organs, it can induce substantial somatic and visceral pain, thereby triggering pronounced neuroendocrine stress responses (9,10). The activation of the hypothalamic-pituitary-adrenal (HPA) axis during surgical stress results in elevated cortisol secretion, disrupted glucose metabolism, and alterations in immune and inflammatory responses (11,12). Therefore, effective postoperative analgesia is imperative to mitigate these adverse physiological effects.

Multimodal analgesia is widely regarded as a superior strategy for postoperative pain management because it combines agents with complementary mechanisms of action to enhance analgesic efficacy while potentially minimizing dose-dependent adverse effects (13,14). Tramadol, a centrally acting synthetic analgesic, exerts its effects through weak μ-opioid receptor agonism and the inhibition of serotonin and norepinephrine reuptake, making it a valuable option for managing moderate postoperative pain (15,16). Previous research suggests that tramadol is both effective and relatively safe for use in rabbits (5,17). Conversely, nonsteroidal anti-inflammatory drugs (NSAIDs) remain a cornerstone of postoperative analgesia by inhibiting cyclooxygenase

activity and reducing prostaglandin synthesis, thereby suppressing inflammation-driven pain (18,19). Flunixin meglumine is among the most frequently used NSAIDs in veterinary medicine and has demonstrated robust analgesic efficacy across various animal species (20).

Beyond the choice of pharmacological agent, the route of administration significantly influences the quality of postoperative pain control. Intraperitoneal (IP) administration may offer localized analgesic effects through direct interaction with peritoneal and visceral nociceptors, while simultaneously contributing to systemic analgesia (21,22). This route has shown promising results for alleviating pain following abdominal surgery in both experimental and clinical settings (23). Nevertheless, there is currently limited comparative evidence regarding the effects of intraperitoneally administered tramadol versus flunixin on the behavioral, physiological, and biochemical markers of postoperative pain and stress in rabbits undergoing ovariohysterectomy.

Therefore, the present study was designed to compare the efficacy of intraperitoneal tramadol and flunixin in modulating postoperative pain and stress responses in rabbits. The evaluation was based on physiological parameters, Rabbit Grimace Scale scores, and specific biochemical indicators, including serum cortisol, glucose, and melatonin levels.

Methods

Study design

This study was designed as a randomized, controlled experimental trial to evaluate and compare the analgesic efficacy of intraperitoneally (IP) administered tramadol and flunixin following ovariohysterectomy in rabbits. The assessment integrated behavioral, physiological, and biochemical indicators of postoperative pain and stress. The experimental protocol adhered strictly to the ARRIVE 2.0 guidelines and international standards for the care and use of laboratory animals (21).

Study location

Surgical procedures, animal housing, and sample collection were conducted at the Department of Surgery, Faculty of Veterinary Medicine. Biochemical serum analyses were performed at the Avin Bonyan Gene Specialized Laboratory, Shahid Chamran University of Ahvaz, Iran.

Animals and housing conditions

Fifteen healthy adult female New Zealand White rabbits (*Oryctolagus cuniculus*), weighing 2135 ± 197 g (Mean ± SD), were enrolled in the study. The animals were sourced from the Razi Vaccine and Serum Research Institute (Karaj, Iran). All rabbits were mature at the time of inclusion and underwent a one-week acclimatization period prior to the commencement of the experiment.

Animals were housed individually in stainless-steel cages under controlled environmental conditions (Temperature: 18-21°C; relative humidity: 55-65%; 12-h light/dark cycle). They were provided with a commercial rabbit pellet diet supplemented with fresh alfalfa hay, with water available *ad libitum* (22).

Experimental groups

Rabbits were randomly allocated into three equal groups (n = 5 per group):

- Control group: Received no intraperitoneal analgesic administration.
- Tramadol group: Received tramadol hydrochloride (5 mg/kg, IP).
- Flunixin group: Received flunixin meglumine (1 mg/kg, IP).

Each treatment was diluted with sterile water for injection to standardize the final administration volume at 2 mL across all groups (23).

Anesthesia and ovariohysterectomy procedure

Ovariohysterectomy was performed using the standard technique described by Olson and Bruce (24). General anesthesia was induced via intramuscular (IM) administration of ketamine hydrochloride (15 mg/kg) and midazolam (3 mg/kg). If necessary, supplemental ketamine was administered via the marginal ear vein to maintain a surgical depth of anesthesia.

Following induction, the ventral abdominal region was clipped and aseptically prepared with 10% povidone-iodine followed by 70% ethyl alcohol. A ventral midline incision of approximately 5 cm was made along the linea alba. Upon entering the abdominal cavity, the ovaries and uterine horns were excised using the standard three-clamp ligation technique. The muscular and peritoneal layers were closed with 3-0 absorbable polyglycolic acid sutures, while the skin was closed with 2-0 non-absorbable nylon.

Intraperitoneal drug administration

Immediately following the removal of the ovaries and uterus and the achievement of complete hemostasis-but prior to final abdominal closure-the assigned treatment was administered via intraperitoneal injection using a sterile insulin syringe. The control group received no IP analgesics.

To prevent postoperative infection, enrofloxacin (5 mg/kg, IM) was administered 24 h after surgery. Additionally, an injectable vitamin B-complex was provided as supportive care according to the postoperative recovery protocol.

Blood sampling and serum preparation

Blood samples were collected from the jugular vein at three time points: baseline (0 h, pre-surgery), and at 2 and 6 h postoperatively. Samples were obtained using sterile 3-mL syringes and transferred into non-anticoagulant tubes. After clot formation at room temperature, the samples were centrifuged at 3500 rpm for 5 min. The resulting serum was aliquoted into sterile microtubes and stored at -20°C until biochemical analysis.

Biochemical assessments

Serum cortisol, melatonin, and glucose concentrations were quantified as biomarkers of postoperative stress and pain. Serum glucose was measured using an automated biochemical analyzer. Serum cortisol and melatonin concentrations were determined using rabbit-specific ELISA kits according to the manufacturer's instructions, with optical density measured via an ELISA plate reader.

Pain assessment

Postoperative pain intensity was evaluated using the Rabbit Grimace Scale (RbtGS), which assesses five facial action units: orbital tightening, cheek flattening, ear position, nose shape, and nostril tension (22). Digital images of each rabbit were captured at 0, 1, 3, 6, 12, and 24 h post-surgery. Scoring was performed by a blinded evaluator who was unaware of the treatment group assignments.

Physiological parameters

Rectal temperature, respiratory rate, and heart rate were recorded at 0, 2, and 6 h post-surgery. Rectal temperature was measured with a digital veterinary thermometer, respiratory rate was determined by observing thoracic excursions, and heart rate was assessed via thoracic auscultation using a stethoscope.

Complete Blood Count (CBC)

To confirm the baseline health status of the animals, a CBC analysis was performed on blood samples collected into EDTA tubes and analyzed with an automated hematology analyzer prior to surgery.

Statistical analysis

Data were analyzed using SPSS version 26.0. The normality of data distribution was verified using the Shapiro-Wilk test. For normally distributed variables, a two-way repeated-measures ANOVA was employed to evaluate the effects of treatment, time, and their interaction. Post hoc analyses were conducted for pairwise comparisons when significant effects were identified. For non-normally distributed variables (e.g., serum cortisol and glucose), non-parametric methods were applied: the Friedman test for within-group temporal comparisons and the Kruskal-Wallis test for between-group comparisons at specific time points. Results are presented as the mean ± standard error (SE), mean ± standard deviation (SD) or median (Interquartile range), as appropriate. The level of statistical significance was set at 0.05.

Results

Pre-experimental findings

Evaluation of hematological parameters before surgery

To confirm the general health status of the animals before the experimental interventions, hematological parameters, including the complete blood count (CBC), were evaluated. The results demonstrated that all measured variables were within the normal physiological reference ranges reported for rabbits.

Mean red blood cell count, hemoglobin concentration, and hematocrit values indicated adequate oxygen-carrying capacity and the absence of anemia in all experimental animals. In addition, erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), remained within normal reference intervals, suggesting normal erythrocyte morphology and hemoglobinization.

Furthermore, total leukocyte count and differential leukocyte populations, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were within the expected physiological ranges, and no evidence of systemic inflammation or active immune response was observed. Platelet counts were also within normal limits, indicating normal hemostatic function in the studied animals.

Overall, the hematological findings confirmed that all rabbits were clinically healthy and met the necessary physiological criteria for inclusion in the experimental study, as shown in Table 1.

General health indicators

Descriptive analysis of physiological parameters

Physiological parameters, including heart rate (HR), respiratory rate (RR), and rectal temperature, were evaluated following ovariohysterectomy. The findings showed a marked increase in mean heart rate during the early postoperative period. The highest HR values were recorded at 2 h after surgery, which may reflect activation of the sympathetic nervous system and physiological responses to surgical stress and acute postoperative pain. Subsequently, a decreasing trend was observed at 6 h post-surgery, suggesting the gradual restoration of physiological homeostasis.

Respiratory rate exhibited a similar pattern, with elevated values detected during the early postoperative phase. Peak RR values were observed at 2 h after surgery, likely reflecting activation of the stress-response axis and increased metabolic demand following the surgical intervention. A relative reduction in RR was subsequently noted at 6 h postoperatively, as shown in Table 2.

Comparison of pharmacological treatments

Comparative analysis of physiological parameters among the experimental groups showed that pharmacological treatment significantly affected both heart rate (HR) and respiratory rate (RR) ($P < 0.001$ for both). Administration of tramadol and flunixin significantly reduced HR and RR values compared with the control group, indicating attenuation of physiological responses associated with postoperative pain and surgical stress.

Among the treatment groups, rabbits receiving tramadol exhibited the lowest mean HR and RR values, suggesting a more effective analgesic response during the early postoperative period. In contrast, no significant differences were observed among the experimental groups with respect to rectal temperature.

Temporal changes in physiological parameters

Evaluation of postoperative changes over time revealed that both HR and RR were significantly influenced by the time elapsed after surgery. The highest values for these parameters were observed at 2 h postoperatively, followed by a declining trend at 6 h after surgery. This pattern reflects the peak of the physiological stress response during the early postoperative phase and the subsequent initiation of physiological adaptation and recovery.

Two-way analysis of variance demonstrated significant effects of treatment, time, and the treatment × time interaction on HR and RR values. However, for rectal temperature, only the effect of time was statistically significant, as shown in Figure 1.

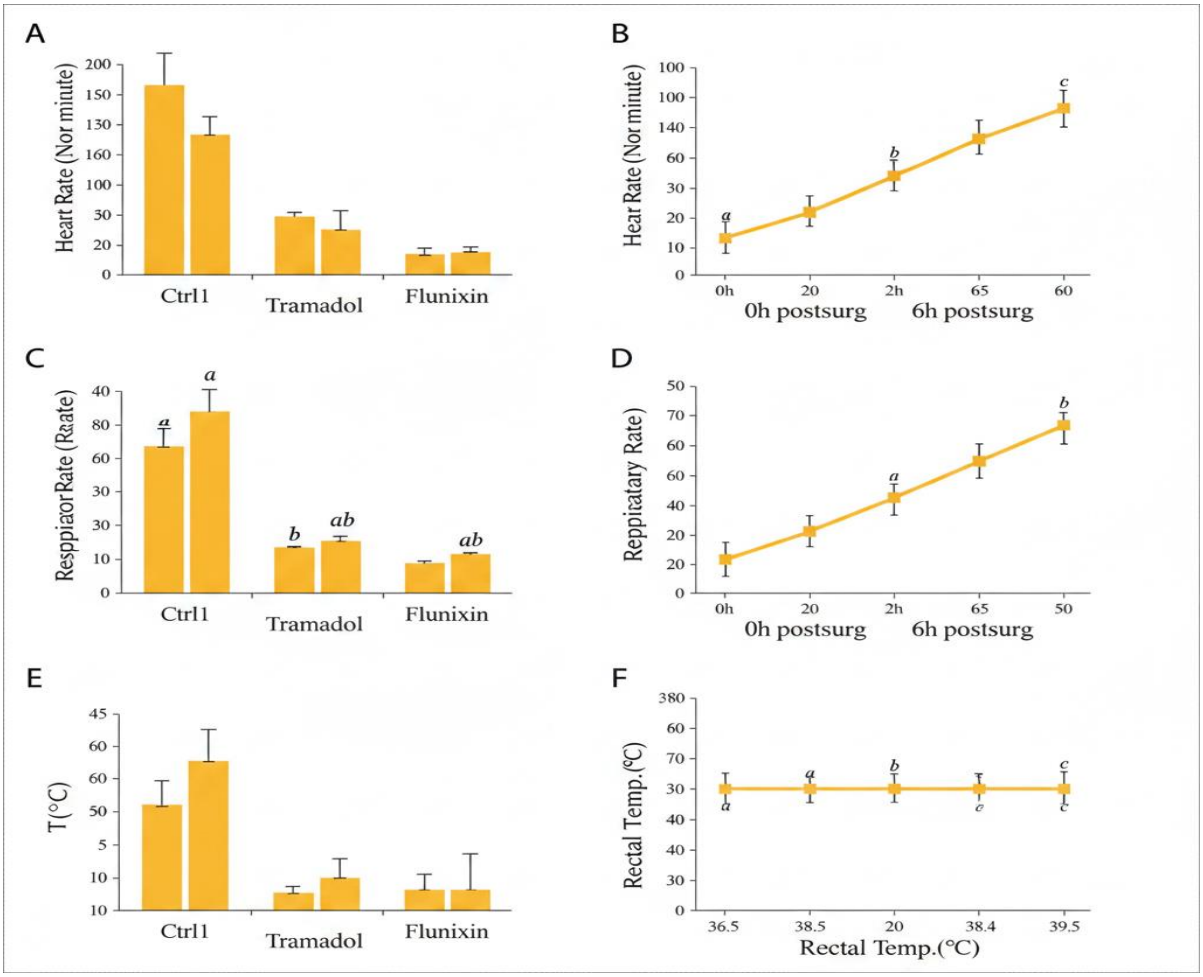

Figure 1. Changes in heart rate, respiratory rate, and body temperature in rabbits undergoing ovariohysterectomy

Changes in physiological parameters in rabbits undergoing ovariohysterectomy. (A) Heart rate in the three experimental groups; (B) changes in heart rate at different postoperative time points; (C) respiratory rate in the three experimental groups; (D) changes in respiratory rate at different postoperative time points; (E) rectal temperature in the three experimental groups; and (F) changes in rectal temperature at different postoperative time points. Data are presented as mean ± standard error (SE).

Table 1. Baseline hematological parameters of rabbits before surgery (N=15)

Variables	Reference Range	Mean ± SD	Min - Max
RBC (10^6/µL)	4.46 - 7.94	6.29 ± 1.06	4.48 - 7.55
HGB (g/dL)	10.4 - 17.4	12.48 ± 1.90	10.50 - 15.90
HCT (%)	35.0 - 50.0	44.12 ± 3.56	36.00 - 49.50
MCV (fL)	50.0 - 75.0	66.23 ± 3.10	61.50 - 72.00
MCH (pg)	18.0 - 24.0	22.03 ± 1.01	18.50 - 23.80
MCHC (%)	27.0 - 34.0	30.67 ± 1.29	28.50 - 33.20
WBC (10^3/µL)	2.0 - 20.0	12.41 ± 5.26	3.90 - 18.50
NEUT (%)	10.0 - 40.0	36.55 ± 9.56	18.00 - 39.50
LYMPH (%)	30.0 - 85.0 *	12.41 ± 5.26	35.00 - 78.00
MONO (%)	0.1 - 0.8	0.84 ± 0.47	0.20 - 0.75
EOS (%)	0.0 - 8.0	2.48 ± 1.22	0.50 - 5.00
BASO (%)	0.4 - 8.0	3.79 ± 2.26	0.50 - 7.50
PLT (10^3/µL)	240 - 600	459.13 ± 133.17	250 - 580

* Reference ranges are based on institutional laboratory standards for New Zealand White rabbits. Values are reported as Mean ± SD.

Table 2. Comparison of physiological parameters among experimental groups and postoperative time points in rabbits undergoing OVH

Variables	Factor	Group/Time	Mean ± SE	95% CI	P-Value (Treatment)	P-Value (Time)
Heart Rate (Beats/Min)	Treatment	T1 (Control)	180.80 ± 7.27	165.19-196.41	< 0.001*	< 0.001*
		T2 (Tramadol)	143.40 ± 2.07	138.97-147.83		
		T3 (Flunixin)	164.60 ± 4.76	154.39-174.81		
	Time	0 h	140.00 ± 1.37	137.06-142.94		
		2 h	179.73 ± 6.04	166.77-192.69		
		6 h	169.07 ± 5.59	157.06-181.07		
Respiratory Rate (Breaths/Min)	Treatment	T1 (Control)	46.53 ± 2.44	41.28-51.77	< 0.001*	< 0.001*
		T2 (Tramadol)	35.80 ± 0.93	33.81-37.78		
		T3 (Flunixin)	41.53 ± 1.32	38.69-44.37		
	Time	0 h	33.73 ± 0.49	32.67-34.78		
		2 h	46.60 ± 1.63	43.09-50.10		
		6 h	43.53 ± 1.83	39.60-47.45		
Rectal Temperature (°C)	Treatment	T1 (Control)	38.66 ± 0.08	38.49-38.83	0.967	< 0.001*
		T2 (Tramadol)	38.68 ± 0.07	38.51-38.84		
		T3 (Flunixin)	38.66 ± 0.08	38.49-38.83		
	Time	0 h	38.34 ± 0.03	38.28-38.40		
		2 h	38.96 ± 0.05	38.86-39.07		
		6 h	38.70 ± 0.04	38.62-38.78		

OVH: Ovariohysterectomy, T1: Control group, T2: Tramadol-treated group, T3: Flunixin-treated group, SE: Standard Error, CI: Confidence Interval
Data are presented as mean ± SE. Statistical significance was assessed using two-way repeated-measures analysis of variance (ANOVA) for the effects of Treatment and Time, with appropriate post-hoc comparisons. *P-Value < 0.05.

Figure 1 illustrates the variations in heart rate (A, B), respiratory rate (C, D), and rectal temperature (E, F) across the experimental groups and postoperative time points. Panels A, C, and E show comparisons among the Control (Ctrl), Tramadol, and Flunixin groups, while panels B, D, and F present the temporal changes in these physiological parameters during the postoperative period. Different lowercase letters (a, b, c) denote statistically significant differences between groups or time points (P-Value < 0.05).

Serum biomarkers

Descriptive analysis of biochemical parameters

Evaluation of serum biomarkers, including cortisol, glucose, and melatonin, showed that surgery induced substantial alterations in stress-related hormonal and metabolic responses. Serum cortisol concentrations increased following surgery and reached their peak levels at 2 h postoperatively, indicating activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to surgical stress.

Concomitant with the elevation in cortisol, serum glucose levels also showed a marked increase, which was likely associated with enhanced gluconeogenesis and elevated metabolic activity under stress conditions.

In contrast, melatonin exhibited a different temporal pattern, with a significant decline observed at 2 h after surgery. This reduction may be attributed to suppression of melatonin secretion during acute stress exposure. However, a considerable increase in melatonin concentration was detected at 6 h postoperatively, potentially reflecting the initiation of regulatory mechanisms and the gradual restoration of physiological balance, as shown in Table 3.

Comparison of treatment effects on serum biomarkers

Comparison of serum biomarker levels among the experimental groups indicated that rabbits receiving tramadol had the lowest cortisol and glucose concentrations and the highest melatonin levels. However, the differences among the groups were not statistically significant for cortisol and glucose. In contrast, a significant treatment effect was observed for melatonin, with the tramadol-treated animals demonstrating higher melatonin concentrations compared with the other experimental groups.

Temporal changes in serum biomarkers

Analysis of temporal trends revealed that cortisol and glucose concentrations peaked at 2 h after surgery, whereas melatonin reached its lowest level at the same time point. At 6 h postoperatively, an inverse pattern was observed, with stress-associated biomarkers decreasing while melatonin levels increased. These findings highlight the important role of postoperative time in modulating hormonal and metabolic responses to surgical stress.

Ovariohysterectomy induced measurable physiological, biochemical, and behavioral responses consistent with postoperative pain and surgical stress in all groups. The intensity of these responses was most evident during the early postoperative period, particularly at 2 h after surgery.

Table 3. Comparison of serum biomarkers (Cortisol, Glucose, and Melatonin) among experimental groups and postoperative time points in rabbits undergoing OVH

Variables	Factor	Group / Time	Median (IQR)	P-Value (Treatment)	P-Value (Time)	P-Value (Treatment × Time)
Cortisol (ng/mL)	Treatment	T1 (Control)	13.0 (9.5-17.0)	0.456	< 0.001*	0.12
		T2 (Tramadol)	11.5 (8.5-14.5)			
		T3 (Flunixin)	14.0 (10.0-18.5)			
	Time	0 h	9.0 (8.0-10.0)			
		2 h	22.0 (19.5-24.5)			
		6 h	8.0 (7.5-8.5)			
Glucose (mg/dL)	Treatment	T1 (Control)	185 (135-235)	0.736	< 0.001*	0.08
		T2 (Tramadol)	170 (140-205)			
		T3 (Flunixin)	200 (140-260)			
	Time	0 h	140 (125-155)			
		2 h	300 (270-335)			
		6 h	124 (118-130)			
Melatonin (pg/mL)	Treatment	T1 (Control)	28.5 (20.5-37.0)	0.022	< 0.001*	< 0.001*
		T2 (Tramadol)	30.0 (25.0-36.0)			
		T3 (Flunixin)	26.0 (19.0-33.5)			
	Time	0 h	35.0 (31.5-38.5)			
		2 h	12.5 (8.5-17.0)			
		6 h	38.0 (37.0-39.5)			

OVH: Ovariohysterectomy, T1: Control group, T2: Tramadol-treated group, T3: Flunixin-treated group, IQR: Interquartile Range
*Data are presented as Median (Interquartile Range). Statistical significance assessed via Kruskal-Wallis test among treatment groups, followed by appropriate post-hoc analyses where applicable. *P-Value < 0.05.

Heart rate and respiratory rate increased after surgery in all groups, indicating activation of the acute stress response. However, rabbits receiving analgesic treatment showed attenuation of these changes compared with the control group. Tramadol appeared to be more effective in reducing the early postoperative physiological responses, especially during the first hours after surgery.

Serum cortisol and glucose concentrations increased postoperatively, with peak values generally observed at 2 h, reflecting activation of the hypothalamic-pituitary-adrenal axis and stress-related metabolic responses. Although the tramadol-treated group showed lower mean cortisol and glucose values than the other groups, the between-group differences in some biochemical variables were not statistically significant.

Melatonin showed a different temporal trend. Its concentration decreased during the early postoperative period, particularly at 2 h after surgery, and then increased at 6 h, which may reflect gradual restoration of neurohormonal balance after the initial stress response.

Behavioral pain assessment using the Rabbit Grimace Scale showed that pain severity was greatest between 3 and 6 h after surgery. Both tramadol and flunixin reduced pain scores compared with the control group; however, flunixin provided more sustained behavioral analgesia during the later postoperative time points, particularly at 6 and 12 h.

Overall, both analgesic protocols mitigated postoperative pain- and stress-related responses compared with the untreated control group. Tramadol demonstrated greater efficacy in controlling early physiological stress responses, whereas flunixin produced a more prolonged effect on behavioral indicators of pain

Statistical analysis of serum biomarkers

Assessment of data distribution indicated that cortisol and glucose values were not normally distributed, whereas melatonin values followed a normal distribution. Accordingly, nonparametric statistical tests were applied for the analysis of cortisol and glucose, while two-way analysis of variance (ANOVA) was used to evaluate melatonin data.

The results demonstrated a significant effect of time on cortisol and glucose levels, whereas treatment type did not exert a statistically significant influence on these parameters. In contrast, melatonin concentrations were significantly affected by treatment, time, and the interaction between these two factors.

Discussion

The present study compared the effects of intraperitoneally administered tramadol and flunixin on postoperative pain and stress responses in rabbits undergoing ovariohysterectomy. The findings showed that both agents reduced surgery-induced physiological and behavioral alterations, although their analgesic profiles differed across postoperative time points. Tramadol was more effective during the early postoperative phase in attenuating physiological stress responses, whereas flunixin provided more sustained control of behavioral pain during later postoperative periods.

Surgical procedures such as ovariohysterectomy are well recognized as potent stressors that activate the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, resulting in changes in heart rate, respiratory rate, endocrine function, and metabolic activity (4,11). In the present study, the highest heart rate, respiratory rate, cortisol, and glucose values were observed during the second postoperative hour, indicating that this interval represented the peak of the acute postoperative stress response. This finding is consistent with previous reports in rabbits and other species showing that the early postoperative period is critical for pain and stress monitoring (5,12).

The greater early efficacy of tramadol in reducing physiological indices may be explained by its central analgesic mechanisms, including weak μ-opioid receptor agonism and inhibition of norepinephrine and serotonin reuptake (15,16). These actions may contribute to a more rapid attenuation of nociceptive transmission shortly after surgery. Previous studies have similarly reported beneficial effects of tramadol on postoperative pain and related physiological responses in rabbits (5,17).

Flunixin also improved postoperative responses compared with the control group, but its principal benefit appeared during the later postoperative phase. As an NSAID, flunixin acts mainly by inhibiting cyclooxygenase activity and reducing prostaglandin synthesis, thereby suppressing inflammation-associated pain (18,20). Because inflammatory mechanisms contribute substantially to pain following abdominal surgery, the prolonged analgesic effect of flunixin observed in the present study is biologically plausible.

With respect to biochemical findings, cortisol and glucose increased after surgery, particularly at 2 h postoperatively, reflecting activation of endocrine and metabolic pathways associated with stress. Although the tramadol group had the lowest mean concentrations of these biomarkers, some between-group differences were not statistically significant. This may be related to the limited sample size and to individual variability in hormonal stress responses. Therefore, these findings should be interpreted cautiously.

Melatonin showed a distinct pattern characterized by an early decline followed by a later increase. This trend may be associated with the disruption of neurohormonal homeostasis during acute surgical stress and subsequent initiation of recovery processes. Importantly, melatonin secretion in rabbits is governed by a pronounced circadian rhythm, with peak levels occurring during the dark phase and a physiological nadir during the light phase. In the current study, surgical procedures and subsequent sampling were performed during the light phase. The observed early postoperative decline likely reflects the immediate suppressive effects of surgical trauma and anesthetic agents

on pineal activity during a period when baseline melatonin is already low. The subsequent increase observed in later hours may represent a physiological rebound or the beginning of the endogenous nocturnal rise, potentially serving an adaptive role due to melatonin's potent antioxidant and anti-inflammatory properties (18,19). Thus, the timing of sample collection is a key determinant in interpreting these fluctuations within the context of the surgical stress response.

Behavioral evaluation with the Rabbit Grimace Scale provided additional evidence regarding analgesic efficacy. Pain scores were highest between 3 and 6 h after surgery, supporting the view that physiological stress and observable pain behavior do not necessarily peak at exactly the same postoperative time. Flunixin showed superior control of behavioral pain during the later time points, which may reflect its stronger action against inflammatory components of postoperative pain.

The intraperitoneal route may have contributed to the analgesic effects observed in this study by allowing local exposure of the peritoneal and visceral tissues to the administered drugs. Previous studies have suggested that this route may improve pain control after abdominal surgery while potentially reducing the need for higher systemic doses (10,23,25). However, further comparative studies are needed to determine the relative contribution of local versus systemic effects.

This study has several limitations. First, the sample size was relatively small, which may have reduced statistical power, particularly for biochemical outcomes. Second, biochemical variables were assessed at only three postoperative time points. More frequent sampling could provide a more detailed profile of the stress response. Third, the study compared single-agent protocols only; future studies should investigate multimodal analgesic regimens combining opioids and NSAIDs.

Overall, the present findings suggest that both tramadol and flunixin can contribute to postoperative pain control after ovariohysterectomy in rabbits, but their effects differ according to postoperative timing and the type of indicator assessed.

Conclusion

Ovariohysterectomy induced clear physiological, biochemical, and behavioral responses associated with postoperative pain and surgical stress in rabbits. Intraperitoneal administration of both tramadol and flunixin reduced these responses compared with the untreated condition, although the pattern of analgesic efficacy differed between the two agents.

Tramadol was more effective in attenuating early physiological stress responses, whereas flunixin provided more sustained control of behavioral pain during the later postoperative period. These findings suggest that both drugs may be useful in postoperative analgesic protocols for rabbits undergoing abdominal surgery. Nevertheless, given the small sample size and limited biochemical sampling points, further studies with larger populations and multimodal analgesic designs are required before broader conclusions can be drawn.

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Ethical Statement

All animal procedures were conducted in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals and were approved by the Research Ethics Committee of the Islamic Azad University, Ahvaz Branch (Approval code: IR.IAU.AHVAZ.REC.1404.394)

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

Author Contributions

All authors contributed to the conception, design, execution, data analysis, and manuscript preparation. All authors read and approved the final version of the manuscript.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence

The authors declare that no artificial intelligence (AI) tools were used in the conception, writing, analysis, or preparation of this manuscript.

References

1. Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, et al. Management of postoperative pain: a clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. J Pain. 2016;17(2):131-57. [View at Publisher] [DOI] [PMID] [Google Scholar]
2. Rawal N. Current issues in postoperative pain management. Eur J Anaesthesiol. 2016;33(3):160-71. [View at Publisher] [DOI] [PMID] [Google Scholar]
3. Glare P, Aubrey KR, Myles PS. Transition from acute to chronic pain after surgery. Lancet. 2019;393(10180):1537-46. [View at Publisher] [DOI] [PMID] [Google Scholar]
4. Pogatzki-Zahn EM, Segelcke D, Schug SA. Postoperative pain-from mechanisms to treatment. Pain Rep. 2017;2(2):e588. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. Campbell M, Tolwani A, Tolwani R, Pacharinsak C, Sharp P, Jampachaisri K, et al. Assessment of postoperative analgesic efficacy and animal well-being using a novel triaxial accelerometer device, the Rodent Fitbit-Like Telemetry Device. J Am Assoc Lab Anim Sci. 2025;64(3):457-67. [View at Publisher] [DOI] [Google Scholar]
6. Weaver LA, Blaze CA, Linder DE, Andrutis KA, Karas AZ. A model for clinical evaluation of perioperative analgesia in rabbits (Oryctolagus cuniculus). J Am Assoc Lab Anim Sci. 2010;49(6):845-51. [View at Publisher] [PMID] [Google Scholar]
7. Pinho RH, Leach MC, Minto BW, Rocha FDL, Luna SPL. Postoperative pain behaviours in rabbits following orthopaedic surgery and effect of observer presence. PLoS One. 2020;15(10):e0240605. [View at Publisher] [DOI] [PMID] [Google Scholar]
8. Benato L, Rooney NJ, Murrell JC. Pain and analgesia in pet rabbits within the veterinary environment: a review. Vet Anaesth Analg. 2019;46(2):151-62. [View at Publisher] [DOI] [PMID] [Google Scholar]
9. Haddad Pinho R, Luna SPL, Esteves Trindade PH, Augusto Justo A, Santilli Cima D, Werneck Fonseca M, et al. Validation of the rabbit pain behaviour scale (RPBS) to assess acute postoperative pain in rabbits (Oryctolagus cuniculus). PLoS One. 2022;17(5):e0268973. [View at Publisher] [DOI] [PMID] [Google Scholar]
10. Puntillo F, Giglio M, Paladini A, Perchiazzi G, Viswanath O, Urits I, et al. Pathophysiology of musculoskeletal pain: a narrative review. Ther Adv Musculoskelet Dis. 2021;13:1759720X21995067. [View at Publisher] [DOI] [PMID] [Google Scholar]
11. Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal route of drug administration: should it be used in experimental animal studies? Pharm Res. 2019;37(1):12. [View at Publisher] [DOI] [PMID] [Google Scholar]

12. Brennan TJ. Pathophysiology of postoperative pain. Pain. 2011;152(3 Suppl):S33-40. [View at Publisher] [DOI] [PMID] [Google Scholar]
13. Akinci SB, Ayhan B, Aycan IO, Tirnaksiz B, Basgul E, Abbasoglu O, et al. The postoperative analgesic efficacy of intraperitoneal tramadol compared to normal saline or intravenous tramadol in laparoscopic cholecystectomy. Eur J Anaesthesiol. 2008;25(5):375-81. [View at Publisher] [DOI] [PMID] [Google Scholar]
14. Boulianne M, Verret M, O'Connor S, Savard X, Neveu X, Marcoux C, et al. Intraperitoneal local anesthetics for postoperative pain management following intra-abdominal surgery: a systematic review and meta-analysis. BMC Anesthesiol. 2025;25(1):235. [View at Publisher] [DOI] [PMID] [Google Scholar]
15. Sauaia A, Moore FA, Moore EE. Postinjury inflammation and organ dysfunction. Crit Care Clin. 2017;33(1):167-91. [View at Publisher] [DOI] [PMID] [Google Scholar]
16. Liu S, Huang H, Zhang C, Chen L, Feng X, Wu Y, et al. Postoperative leukocyte counts as a surrogate for surgical stress response in matched robot- and video-assisted thoracoscopic surgery cohorts of patients: a preliminary report. J Robot Surg. 2024;18(1):176. [View at Publisher] [DOI] [PMID] [Google Scholar]
17. Fruk Šimunec S, Žura Žaja I, Pejaković Hlede J, Shek Vugrovečki A, Ružić A, Đuričić D. Cortisol as a biomarker for stress: a review of sampling methods in small ruminants. Croat Vet J. 2026;57(3):57-2. [View at Publisher] [DOI] [Google Scholar]
18. Caroprese M, Albenzio M, Marzano A, Schena L, Annicchiarico G, Sevi A. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. J Dairy Sci. 2010;93(6):2395-403. [View at Publisher] [DOI] [PMID] [Google Scholar]
19. Xie Z, Chen F, Li WA, Geng X, Li C, Meng X, et al. A review of sleep disorders and melatonin. Neurol Res. 2017;39(6):559-65. [View at Publisher] [DOI] [PMID] [Google Scholar]
20. Maitra S, Baidya DK, Khanna P. Melatonin in perioperative medicine: current perspective. Saudi J Anaesth. 2013;7(3):315-21. [View at Publisher] [DOI] [PMID] [Google Scholar]
21. Vedantam D, Poman DS, Motwani L, Asif N, Patel A, Anne KK. Stress-induced hyperglycemia: consequences and management. Cureus. 2022;14(7):e26714. [View at Publisher] [DOI] [PMID] [Google Scholar]
22. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000410. [View at Publisher] [DOI] [PMID]
23. Flecknell PA. Laboratory animal anaesthesia. 4th ed. Amsterdam: Academic Press; 2016. [View at Publisher]
24. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci. 2011;50(5):600-13. [View at Publisher] [PMID] [Google Scholar]
25. Bencharif D, Amirat L, Garand A, Tainturier D. Ovariohysterectomy in the bitch. Obstet Gynecol Int. 2010;2010:542693. [View at Publisher] [DOI] [PMID] [Google Scholar]

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