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Comparison of intraperitoneal medetomidine and paraincisional bupivacaine on post-operative pain management of ovariohysterectomy in dogs

Seyed Mohamad Sajjadi Dezfouli[1], Ali Baniadam[2], Soroush Sabiza[2*] and Seyedeh Misagh Jalali[2]

[1] DVSc Student in Veterinary Surgery, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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Abstract

Providing effective pain relief after surgery in veterinary medicine is a crucial aspect of ethical and clinical care, particularly during procedures like spaying. Various analgesic drugs have been used for this purpose, but the management of analgesia in different animals during and after surgery requires different strategies. In this study, the effect of medetomidine and bupivacaine with different methods of administration in ovariohysterectomy surgery was investigated in bitches. Twenty-Five native breed bitches (1-4 years, 15-25 kg) were divided into 5 groups of 5 based on the type of drug and the method of administration: control group, medetomidine IM, medetomidine IP, medetomidine IP and bupivacaine SC, and bupivacaine SC alone. In all groups, defined drugs for each group were administered at three stages: prior to the skin incision, simultaneously with ligation of the first ovarian pedicle, and before suturing the *linea alba*. Before, during and after surgery, sedation quality, pain quality, anesthesia depth, recovery quality, vital signs (body temperature, respiratory rates, cardiovascular parameters), and biochemical, parameters were measured at predetermined times. After surgery, analgesia was measured by Simple Descriptive Scores (SDS), Visual Analogue Scale (VAS), University of Melbourne Pain Scale (UMPS), and Glasgow Composite Measure Pain Scale-Short Form (CMPS-SF) tests. The obtained data were analyzed with SPSS software and appropriate statistical tests. The results indicated that administering the same dose of intramuscular medetomidine compared to intraperitoneal medetomidine resulted in significant differences in pain (measured by CMPS-SF and VAS test), heart rate, and cortisol levels at specific times after surgery. Administering bupivacaine alone significantly reduced surgical pain and decreased recovery time compared to administering medetomidine alone or in combination with bupivacaine. Animals receiving intraperitoneal medetomidine required a rescue dose, while no rescue dose was needed in other groups. The doses used in all groups did not disrupt the animals' physiological functions, and cardiovascular, respiratory, and rectal temperature parameters remained within the normal range. The activity of serum enzymes related to general tissue integrity also stayed within the normal range. Evaluation of pain using VAS, UMPS, and CMPS-SF methods did not show a preference for the effectiveness of administering the same dose of intraperitoneal medetomidine over intramuscular medetomidine (alone or with bupivacaine at the surgical incision) for managing pain, recovery after surgery, and physiological parameters of cardiovascular, respiratory, and rectal temperature.

Key words: Pain management, Ovariohysterectomy, Female dogs, Medetomidine, Bupivacaine

Introduction

Sneddon (2009) defines pain in animals as follows: "The perception and aversive sensory experience of a noxious stimulus associated with potential or actual injury"

[*] **Corresponding Author**: Soroush Sabiza, Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: s.sabiza@scu.ac.ir

(Walters, 2018). Pain is classified into physiological and pathological types. Physiological pain arises without tissue damage, alerting the animal to potential harm. Pathological pain occurs following tissue and nerve damage; its intensity may vary depending on the severity and speed of tissue injury (Kania et al, 2021). Following tissue destruction, a series of local and systemic responses such as inflammation are created in the organism. Inflammation occurs following a chain of organized cellular and vascular responses that include the secretion of a group of inflammatory mediators and signaling molecules (such as histamine, prostaglandin, and leukotriene, free radicals derived from oxygen, nitrogen, and serotonin) by immune defense cells (Abdulkhaleq et al, 2018). Analgesics are essential for managing pain in veterinary medicine, most commonly for postoperative pain and also for various other painful conditions. If the administration of analgesics is ignored in animals, an increase in stress and its related consequences, i.e. an increase in norepinephrine, epinephrine, cortisol, vasopressin, renin, angiotensin II, aldosterone, ACTH, glucose, and a decrease in insulin and testosterone levels occur (Kania et al, 2021). Alpha-2 receptor agonist drugs, such as medetomidine, in the cortical layer of the brain, brain stem, and spinal cord are used as sedatives and hypnotics in pre-anesthesia protocols. The combination of medetomidine with some drugs like alfaxalone has been used to induce sedation or pre-anesthesia in dogs and cats (Kamohara et al, 2022). Medetomidine is associated with side effects such as hyperglycemia, pulmonary edema, bradycardia, and decreased blood pressure, cardiac output, body temperature and increased cardiac afterload (Raekallio et al, 2017). Among the reasons for surgery and the need for anesthesia in animals is ovariohysterectomy to control the population, prevent diseases of the reproductive system, and weaken unpleasant behaviors related to sex hormones. The mild to moderate pain experienced by the animal in this surgery requires appropriate analgesics. Therefore, it is necessary to determine the preference for using the analgesics from an economic and ethical point of view (Rezaeipour et al, 2022).

Intraperitoneal administration of local anesthetics is a valuable and validated method for pain control after abdominal surgery. Due to capillary structures in the peritoneum and wide surfaces for drug exchange between the peritoneum and plasma, this part is used in medicine and veterinary medicine along with intravenous, intramuscular, and subcutaneous methods. In this method, before suturing the *linea alba*, a local anesthetic or analgesic is splashed on the viscera. Intraperitoneal administration of lidocaine or bupivacaine has been shown to provide adequate analgesia after ovariohysterectomy surgery in dogs (Chilkoti et al, 2019). In a study, medetomidine was used as an intraperitoneal infusion to control pain after laparotomy in pregnant sheep, and its effects in creating a continuous plasma concentration within 10 hours; also, controlling appropriate pain after surgery without other analgesic compounds have been proven (Murdoch et al, 2013). In human medicine, intraperitoneal administration of dexmedetomidine (an active isomer of medetomidine) along with local anesthetics such as ropivacaine and bupivacaine has been used for analgesia after laparoscopy, and its effectiveness was reported (Kandi et al, 2022).

Since ovariohysterectomy is one of the most common surgeries in spaying a bitch and pain management is important in this type of surgery, this study aims to investigate the effectiveness of intraperitoneal and/or intramuscular administration of medetomidine at the same time as the administration of bupivacaine at the surgical site for pain management after ovariohysterectomy.

Materials and methods

Animals

Twenty-five adult female dogs of native breed (aged 1-4 years and weighing 15-25 kg) were selected and examined by a small animal veterinary specialist for signs of inflammation or infection. A blood test (Complete Blood Count (CBC), total protein) was done to confirm their health status. A wet diet included canned dog food and clean water available to all dogs. The dogs were acclimated to the new environment and people involved in the study for seven days. Subsequently, they were randomly divided into five groups of 5 based on the type of drug and the method of administration.

1. Control Group: After aseptic preparation of the surgical site and prior to the skin incision, saline (0.2 ml/kg, SC) was injected into the subcutaneous tissue around the incision site; saline (0.9%, IM) was injected simultaneously with ligation of the first ovarian pedicle; saline (0.2 ml/kg, IP) was splashed into the peritoneal cavity before suturing the *linea alba*.

2. Medetomidine IM Group: After aseptic preparation of the surgical site and prior to the skin incision, saline (0.2 ml/kg, SC) was injected into the subcutaneous tissue around the incision site; Medetomidine (20 µg/kg, IM) was injected simultaneously with ligation of the first ovarian pedicle; saline (0.2 ml/kg, IP) was splashed into the peritoneal cavity before suturing the *linea alba*.

3. Medetomidine IP Group: Saline (0.2 ml/kg, SC) was injected into the surgical incision sites; saline (0.9%, IM) was injected simultaneously with ligation of the first ovarian pedicle; Medetomidine (20 µg/kg, IP) was splashed into the peritoneal cavity before suturing the *linea alba*.

4. Medetomidine IP and Bupivacaine SC Group: Bupivacaine (1mg/kg, SC) was injected into the surgical incision sites; saline (0.9%, IM) was injected simultaneously with ligation of the first ovarian pedicle; Medetomidine (20 µg/kg, IP) was splashed into the peritoneal cavity before suturing the *linea alba*.

5. Bupivacaine SC Group: Bupivacaine (1mg/kg, SC) was injected into the surgical incision sites; saline (0.9%, IM) was injected simultaneously with ligation of the base of the first ovarian pedicle; saline (0.2 ml/kg, IP) was splashed into the peritoneal cavity before suturing the *linea alba*.

Preparation of animal before surgery

Access to the food and water was restricted 12 and 2 hours before surgery, respectively. The hair over the cephalic veins in both forelimbs was shaved,–and a peripheral venous catheter (20 gauges) was inserted in both cephalic veins for administration of the fluids and blood sampling. Sedation was induced with Acepromazine (0.05mg/kg, IM) and Morphine (0.5mg/kg, IM) (Lambertini et al, 2018).

The skin from the xiphoid to the pubis and laterally to 10 cm on either side of the ventral midline was clipped. Cefazolin (22 mg/kg, IV) was administered as prophylaxis. Five minutes before induction of general anesthesia, oxygen was provided via a flow-by system. Anesthesia was induced with propofol (6 mg/kg, IV) and titrated until the jaw was easily opened and intubation was possible. Dogs were positioned in dorsal recumbency and the monitoring leads were attached. Anesthesia was maintained with an inhalation anesthesia machine equipped with an isoflurane vaporizer (concentration 1.5%) and an oxygen flow of 1.5 liters. Ringer's lactate solution (5 ml/kg/hour) was infused intravenously throughout the surgery. The surgical area was prepared aseptically with betadine scrub (7.5%) and chlorhexidine (2%).

Surgical Technique

All the procedures of this study were performed by a fixed three-member team, one surgeon, and two non-scrubbed assistant (blinded to the allocation) for blood sampling and data recording during and after surgery. The evaluations were carried out in specific timings that started 5 minutes before the induction of anesthesia

(T0) and ended (T11) 6 hours after the completion of the surgery. Based on this, the time of skin incision (T1), *linea alba* incision (T2), ligature of the first ovarian pedicle (T3), skin suture (T4), and 30, 60, 120, 180, 240, 300, 360 minutes (T5-T11) were defined after the completion of surgery. Blood samples were collected at baseline (T0), skin incision (T1), skin closure (T4), and at 2, 6, and 24 hours post-surgery (T7, T11, and T24, respectively) in separate tubes. During the ovariohysterectomy surgery, the required parameters were obtained by the monitoring device and recorded in specific forms.

Ovariohysterectomy

The main surgical procedure was performed from the midline approach. First, using a scalpel blade (number 15), an incision of about 10 cm was made on the lower skin surface of the umbilical scar. The linea alba was exposed with Metzenbaum scissors and then incised. The peritoneal cavity was subsequently accessed (Bencharif et al, 2010).

Using a spay hook, the left ovary was searched and revealed, then the suspensory ligament was transected. After creating a window in the broad ligament of the uterus (close to the ovarian pedicle), the hemostat forceps were placed on the ovarian pedicle. The vessels were ligated with the polyglycolic acid synthetic absorbable sutures (number 0) and transected with scissors. Following confirmation of the hemostasis, the ovarian pedicle was released into the abdominal cavity. These steps were repeated for the right ovary. Lastly, the uterine body (caudal to the uterus horns) was double ligated and excised cranial to the cervix. After ensuring that there was no bleeding, it was released into the abdominal cavity.

The surgical incision was closed in three layers:

Linea alba: simple continuous suture with 0 PGA suture material

Subcutaneous tissue: simple continuous suture with 3-0 PGA suture material

Skin: cruciate pattern suture with 3-0 non-absorbable nylon suture material

The time of the final suture was recorded, and the surgical site was dressed.

Evaluations of some parameters during and after surgery

Evaluation of the quality of sedation: After 30 minutes of intramuscular injection of sedative drugs, the quality of sedation was scored from 0 to 3. Score 0: no sedation. Score 1: mild, Score 2: moderate sedation. Score 3: deep sedation (Mair et al, 2009).

Assessment of the pain quality and depth of anesthesia: The following method was used to evaluate the rate of painful stimuli after anesthesia induction and tracheal intubation. A Rochester-Pean hemostatic forceps was used to apply pressure on the second toe joint of the left hind foot for 30 seconds or until the animal withdrew the foot. The degree of response to stimulation was scored based on the following grading: 0: No response; 1: Weak and low movement; 2: Retraction of limbs; 3: Limb retraction accompanied by head elevation (Muir et al, 2009).

Recovery Quality Evaluation: This parameter was evaluated from stopping the anesthetic to standing the animal completely and scored as follows, 1: Lying on the sternum with low or no struggle, standing and walking without a problem or with the tiniest trouble. 2: Low struggle needs help to lay on the sternum and stand, respond to external stimuli, and silence the animal after lying on the sternum. 3: Struggle for a long time, difficulty on the sternal positioning and standing, enhance the feet and taps for a long time when helping the animal (Muir et al, 2009).

Duration of surgery*:* This parameter was recorded from the start of the skin incision to the last skin suture.

Duration of anesthesia: It was recorded from the time of the tracheal intubation to the time the patient was standing.

Vital signs: Rectal temperature; Respiratory rate, percentage of hemoglobin oxygen saturation (SpO_2), End-tidal CO_2 ($EtCO_2$); Cardiovascular indicators including systolic

blood pressure, diastolic blood pressure, mean arterial blood pressure, heart rate and capillary refill time (CRT). The parameters were measured after tracheal intubation by a multi-parameter monitoring device, and a capnograph machine (Drager Company, Lubeck, Germany). The CRT was assessed by applying pressure on the gingival mucosa.

In order to evaluate the cardiovascular effects of medetomidine, heart rate, CRT, SpO_2, systolic, diastolic, and mean arterial blood pressure were measured at 5, 10, 15, and 20 minutes after the intramuscular or peritoneal administration of medetomidine.

Blood Tests: Serum glucose, and cortisol levels, along with lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), and Creatine kinase (Bussen et al.) activity were measured. at T0, T1, T4, and T7, T11, and T24.

To measure the level of glucose and the activity of CK, LDH, and AST enzymes, a commercial kit (BiorexFars, Fars, Iran) and an autoanalyzer (BT-1500 biochemical analyzer, Italy) were applied. The competitive ELISA method was used to measure cortisol concentration according to the manufacturer's instructions for the commercial kit (Monobind Company, USA).

Analysis of analgesia after surgery: We used different methods to investigate the postoperative analgesia; simple descriptive scores (SDS), Visual Analogue Scale (VAS), University of Melbourne Pain Scale (UMPS), and Glasgow Composite Measure Pain Scale-Short Form (CMPS-SF) at 0, 15, and 30 minutes and then at 1, 2, 3, 4, 5, and 6 hours after the surgery (Baniadam et al, 2021; Lambertini et al, 2018).

statistical analysis

The SPSS software version 24 (IBM Corporation, NY, USA) was used to analyze the data. One-way ANOVA (analysis of variance) with Tukey's post hoc test was utilized to compare the data obtained from the heart rate, respiration rate, arterial blood pressure, rectal temperature, plasma glucose, and cortisol levels among the groups. Repeated measures ANOVA was employed to evaluate these parameters within the groups over time. The Kruskal-Wallis test was used to compare the data obtained from pain assessment among the groups. Friedman's test assessed differences within the groups for these parameters. Results were presented as mean ± standard error. For qualitative parameters, results were presented as median (minimum-maximum), with $p<0.05$ values considered significant in all statistical analyses.

Results

Weight, Length of surgical incision, Duration of surgery and Duration of anesthesia

Among the parameters of mean weight, length of surgical incision, duration of surgery, and duration of anesthesia in each of the studied groups, only the duration of anesthesia showed a statistically significant difference between groups (P=0.005). However, there was no statistically significant difference in other parameters (Table 1).

Table 1: Mean ± Standard deviation of weight, length of surgical incision, duration of surgery and duration of anesthesia in different groups (n=5)

Group	Weight (kg)	Surgery duration (min)	Anesthesia duration (min)	Length incision (cm)
Control	23.10±6	31.33± 6	88.7±41	6.94±0.65
Med-IM	23.17±5	31.45±5	195.98±53 [a]	6.93± 0.54
Med-IP	23.45±2.3	29.67±2	205.64±52 [a]	6.87± 0.44
Med-Bup	24.64±3	27.69±5	190.12±66 [a]	6.17± 0.44
Bup-SC	22.76±4.5	29.86±2	113.13±30	6.18± 0.27

Med: Medetomidine; IM: Intramuscular; IP: Intraperitoneal; SC: Subcutaneous; Bup: Bupivacaine.
[a] Significant difference with the control group (P=0.005)

Anesthesia Parameters

Among the parameters of anesthesia, there was a statistically significant difference in terms of recovery quality in each of the studied groups. The quality of recovery in the medetomidine groups was poorer compared with the Bup-SC group and the control group (P<0.05) (Table 2).

Table 2: Mean ± standard deviation of sedation, depth of anesthesia and recovery quality in different groups (n=5)

Groups	Sedation quality	Depth of anesthesia	Recovery quality
Control	2.4±0.5	0.8±0.5	1.6±0.5
Med-IM	2.4±0.5	0.8±0.4	2.8±0.4[a]
Med-IP	2.4±0.5	1.4±0.5	3±0[a]
Med-Bup	2.4±0.9	0.6±0.5	3±0[a]
Bup-SC	2.4±0.5	0.4±0.3	1.4±0.5

Med: Medetomidine; IM: Intramuscular; IP: Intraperitoneal; SC: Subcutaneous; Bup: Bupivacaine.
[a]Significant difference with the control group (P<0.05)

Vital Parameters

Cardiovascular Parameters

The data analysis did not reveal a statistically significant difference in the *Cardiovascular Parameters* between the groups (Table 3). Changes in *Cardiovascular Parameters* at different time points among the groups were also not significant [F(6,36)=0.6, P=0.72].

There was a significant increase in diastolic blood pressure in the Med-IM and Med-Bup groups at T4 compared to the control group. Additionally, in the Med-Bup group at this time, there was a significant increase in diastolic pressure compared to the Med-IP group (Table 3). The mean diastolic blood pressure in the groups receiving medetomidine after five-minute intervals showed a statistically significant difference. In the Med-Bup group, at 15 minutes after the administration of medetomidine, there was a significant increase in diastolic blood pressure compared to the Med-IP group (P=0.028) (Table 5).

A significant increase in the mean arterial blood pressure was observed in the Med-IM (P=0.019) and Med-Bup (P=0.045) groups at T4 compared to the control group (Table 3).

A significant decrease in the mean heart rate was observed in the Med-IM (P=0.014), Med-IP (P=0.005), and Med-Bup (P=0.001) groups at T4, as well as in these same groups at T5 (P=0.001) and T7 [Med-IM (P=0.003), Med-IP (0.038), Med-Bup (P=0.026)] compared to the control group. Additionally, at T3, the Med-IP and Med-Bup groups showed a significant decrease (P=0.022) compared to the Med-IM group (Table 3). The mean heart rate at different time points in various groups exhibited statistically significant changes [F (22,110) = 3.03, P<0.0001]. Fluctuation in the mean heart rate in the groups receiving medetomidine at five-minute intervals after administration showed a significant decrease in the Med-IP and Med-Bup groups (P=0.022) compared to the Med-IM group (Table 5).

Capillary refill time (CRT) was within the normal range in all groups. The results showed statistically significant differences in this factor at T2 and T3 times among the groups. The CRT was lower in the medetomidine-administered groups compared to the control group (Table 3). Changes in the CRT over time in different groups were not significant [F (12, 63) =1.2, P=0.30].

Respiratory Parameters and Rectal Temperature

The percentage of hemoglobin saturation with oxygen (SpO_2) was within the normal range in all groups, but there were differences observed in some groups. At T6, there was a significant decrease in the Med-IM group compared to the control group (P=0.018). At T10, the percentage of SpO_2 was significantly decreased (P=0.044) in the Med-Bup and Bup-SC groups compared

to the control group (Table 4). The mean fluctuations of SpO2 over time in different groups were not statistically significant [F(14,70)=1.07, P=0.39].

There was no statistically significant difference between groups in EtCO2 (Table 4). Fluctuations in EtCO2 over time in different groups also showed no statistically significant difference [F(7.5,37)=0.55, P=0.79].

In all groups, the mean breathing rate was within the normal range, but differences were observed in some groups. At T6, there was a statistically significant decrease in breathing rate in the Med-IP group compared to Bup-SC (P=0.039) (Table 4). Fluctuations in the number of breaths per minute overtime in different groups were not significant [F(22,114)=0.95, P=0.53].

The mean rectal temperature in the Med-Bup and Med-IP groups at T4 (P=0.019) and T6 (P=0.03) increased significantly compared to the control group (Table 4). However, changes in body temperature at different times across various groups did not show any statistically significant differences [F (27, 138)=1.5, P=0.069].

Pain Assessment

Pain evaluation using the SDS method at different times in different groups did not show significant differences among the groups (Figure 1). However, there was a significant variation in pain scores over time within each group at the specified intervals [$\chi 2$= 35; P<0.0001]. The highest median pain score was recorded at T7 in the Med-IM and Med-IP groups.

When evaluating the pain by using the VAS method, there was a statistically significant difference in pain ratings among the groups (P<0.05). At T6, median pain score in the Med-IM group was significantly lower than control, Med-IP and Bup-SC; but difference between Med-IM and Med-bup was not significant (P>0.05). At T7 and T8, the pain rating in Bup-SC group was lower compared to the control, Med-IM and Med-IP groups (P<0.05) (Figure 2). No statistically significant difference was observed between groups at T9-T11. There was also a statistically significant difference in pain rating fluctuations over time among the groups [$\chi 2$=91; P<0.0001].

Furthermore, pain evaluation using the UMPS method, a statistically significant difference was observed in pain ratings among the groups (P<0.05). Specifically, at T6, the pain rating in the Med-IM, Med-Bup and Bup-SC groups was significantly lower than the control group. The difference between Med-IP and control group was not significant at this time (P>0.05). Additionally, the median pain score at T7 in the Med-Bup and Bup-SC groups was lower than the control group, but this difference was not statistically significant (P>0.05) (Figure 3). No statistically significant difference was observed between groups at T8-T11. Similar to the VAS method, a statistically significant difference was observed in pain rating fluctuations over time among the groups [$\chi 2$= 93; P<0.0001].

There was a statistically significant difference in pain ratings among the groups in the CMPS-SF (P < 0.05). At T6, median pain score in the Med-IM group was significantly lower than other groups (P<0.05). At T7, no statistically significant difference was observed between groups. At T8, the median pain score in the Bup-SC group was significantly lower than control, Med-IM and Med-IP; but, at this time, median pain score in the Med-Bup group was lower than Bup-SC significantly (P < 0.05) (Figure 4). No statistically significant difference was observed between groups at T9-T11. A statistically significant difference was observed in the changes in pain ratings over time in different groups [$\chi 2$ = 108; P < 0.0001].

During the experiment, three dogs in the control group and two dogs in the Med-IP group received a dose of morphine (0.5 mg/kg, IM) as a rescue dose after surgery. None of the groups receiving analgesia showed significant changes in the physiological parameters investigated in this study.

Table 3: Mean ± standard deviation of cardiovascular parameters in different groups at different times (n=5)

Variable	Groups	T0 (a)	T1 (b)	T2 (c)	T3 (d)	T4 (d)	T5 (e)	T6 (f)	T7 (g)	T8 (h)	T9 (i)	T10 (j)	T11 (k)
Systolic arterial pressure (mmHg)	Control (A)	119 ±11	89 ±14	96 ±9	102 ±14	110 ±23	121 ±15	134 ±26	126 ±15	140 ±18	132 ±8	140 ±17	141 ±13
	Med-IM (B)	129 ±6	106 ±23	105 ±15	120 ±14	123 ±10	112 ±21	113 ±16	122 ±25	126 ±21	143 ±18	132 ±13	139 ±18
	Med-IP (C)	118 ±13	99 ±10	97 ±13	104 ±22	109 ±9	108 ±10	122 ±14	129 ±12	120 ± 12	130 ±27	143 ±19	131 ±7
	Med-Bup (D)	129 ±19	102 ±5	98 ±13	119 ±9	113 ±9	108 ±22	110 ±11	128 ±7	132 ±16	131 ±22	129 ±10	132 ±14
	Bup-SC (E)	118 ±20	96 ±16	100 ±18	120 ±10	101 ±10	127 ±9	125 ±15	136 ±22	133 ±10	134 ±14	134 ±7	134 ±28
Diastolic arterial pressure (mmHg)	Control (A)	63 ± 8	43 ±5	46 ±3	64 ±23	55± 14 B,D	77± 11	80 ±16	69 ±15	82 ±22	85 ±19	84 ±16	87 ±11
	Med-IM (B)	69 ± 12	47 ±18	47 ±15	68 ±23	79± 10A	80± 16	79 ±24	87 ±17	90 ±10	97 ±21	83 ±12	92 ±12
	Med-IP (C)	70 ± 20	44 ±11	45 ±8	58 ±14	61± 6D	74± 11	79 ±15	87 ±7	85 ±4	79 ±15	83 ±11	74 ±12
	Med-Bup (D)	76 ± 16	45 ±8	47 ±5	67 ±14	79± 6A,C	73± 18	76 ±18	86 ±7	79 ±6	79 ±18	77 ±11	78 ±25
	Bup-SC (E)	66 ±7	40 ±6	45 ±11	69 ±3	42±8	69± 23	80 ±17	91 ±9	76 ±14	87 ±8	90 ±11	92 ±13
Mean arterial pressure (mmHg)	Control (A)	88 ±7	60 ±19	67 ±9	79 ±21	69±13 B,D	90 ±9	93 ±19	89 ±10	97 ±23	99 ±19	99 ±15	98 ±17
	Med-IM (B)	89 ±14	62 ±19	63 ±15	84 ±20	91± 15A	90± 14	93 ±14	99 ±19	98 ±13	108 ±13	96 ±10	106 ±11
	Med-IP (C)	89 ±14	62 ±9	62 ±3	72 ±17	76±2	85 ±9	89 ±13	102 ±5	96 ±6	96 ±18	101 ±18	89 ±9
	Med-Bup (D)	94 ±13	61 ±1	57 ±10	84 ±11	89±2 A	87± 17	87 ±14	98 ±6	91 ±9	92 ±17	93 ±9	94 ±19
	Bup-SC (E)	84 ±8	58 ±8	63 ±14	87 ±8	62±8	93± 12	94 ±15	107 ±14	90 ±12	99 ±8	107 ±2	104 ±17
Heart rate (beats per minute)	Control (A)	109 ±26	91 ±55	104 ±27	95 ±21	126± 29 B,C,D	146 ±62 B,C,D	128 ±64	114 ±34 B,C,D	105 ±43	99 ±31	100 ±31	99 ±25
	Med-IM (B)	104 ±9	97 ±6	101 ±3	86± 6C,D	81± 11A	52± 16 A	51 ±14	53± 12A	62 ±9	82 ±16	85 ±11	93 ±9
	Med-IP (C)	96 ±21	80 ±17	77 ±27	72 ±7B	75± 13A	54± 14A	63 ±20	69± 23A	75 ±17	85 ±24	89 ±22	88 ±10
	Med-Bup (D)	96 ±13	76 ±15	77 ±16	69± 12B	66± 24A	55± 8A	62 ±13	67± 12A	82 ±12	78 ±13	80 ±14	82 ±7
	Bup-SC (E)	104 ±22	100 ±24	92 ±18	93 ±17	102 ±14	95± 20	108 ±51	92± 23	97 ±26	108 ±20	107 ±21	107 ± 24
Capillary refill time (second)	Control (A)	1.8 ±0.44	1.4± 0.54	1.6± 0.54 B,C,D	1.6± 0.54 B,C,D	1.4± 0.54	1.4± 0.54	1.4± 0.54	1.4± 0.54	1.2± 0.44	1±0	1±0	1±0
	Med-IM (B)	1±0	1±0	1± 0A	1± 0A	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0
	Med-IP (C)	1.2± 0.44	1±0	1± 0A	1± 0A	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0
	Med-Bup (D)	1.4± 0.54	1.2± 0.44	1± 0A	1± 0A	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0
	Bup-SC (E)	1.4± 0.54	1.2± 0.44	1.2± 0.44	1.2± 0.44	1.2± 0.44	1.2± 0.44	1±0	1±0	1±0	1±0	1±0	1±0

Before induction of anesthesia (T0), Time of skin incision (T1), *Linea alba* incision (T2), Ligation of the first ovarian pedicle (T3), Skin suture (T4), and 30, 60, 120, 180, 240, 300, 360 minutes after the completion of surgery (T5-T11).

Table 4: Mean ± standard deviation of respiratory parameters and rectal temperature in different groups at different times (n=5)

Variables	Groups	T0 (a)	T1 (b)	T2 (c)	T3 (d)	T4 (d)	T5 (e)	T6 (f)	T7 (g)	T8 (h)	T9 (i)	T10 (j)	T11 (k)
SPO_2 (%)	Control (A)	96.8 ±1	97.4 ±2	96.8 ±2	96.2 ±3	95.4 ±3	94.4 ±2	97.4 ±1B	95.8 ±1	96.6 ±1	97.2 ±1	97.2± 1D,E	97.6 ±1
	Med-IM (B)	97.8 ±0 E	94.4 ±5	95.4 ±5	93.4 ±5	93.4 ±2	90.2 ±2	92.4 ±2 A	94±2	95.6 ±1	96.8 ±1	97.4 ±0	97±0
	Med-IP (C)	95.6 ±2	95.8 ±2	96.2 ±1	94.6 ±2	97±2	93.4 ±4	94.4 ±2	94.6 ±1	95.4 ±1	95.8 ±0	96±1	96.6 ±1
	Med-Bup (D)	96.2 ±1	96.2 ±3	97±2	92.4 ±6	95.4 ±3	91.8 ±2	94.4 ±2	97±1	97.2 ±1	95.2 ±2	94.4 ±2 A	96.4 ±1
	Bup-SC (E)	94.4 ±1 B	96.2 ±1	95.6 ±3	94.6 ±2	97±2	93.6 ±1	94.6 ±2	93.8 ±1	95.8 ±1	96.4 ±1	94.4 ±0 A	95.6 ±0
$EtCo_2$ (mmHg)	Control (A)	-	25 ±1	24.8 ±2	25.4 ±3	25.8 ±3	-	-	-	-	-	-	-
	Med-IM (B)	-	25.4 ±5	26.2 ±4	27 ±3	28.8 ±4	-	-	-	-	-	-	-
	Med-IP (C)	-	24.4 ±1	26.2 ±3	26.6 ±2	30.4 ±6	-	-	-	-	-	-	-
	Med-Bup (D)	-	23.2 ±8	26.2 ±3	25.4 ±3	29.4 ±4	-	-	-	-	-	-	-
	Bup-SC (E)	-	18 ±8	22.8 ±6	23±6	25.2 ±5	-	-	-	-	-	-	-
Respiratory Rate (breath per minute)	Control (A)	39± 14	34± 18	28± 19	23±4	19±8	24±5	24±4	24±4	24±7	25±6	24±5	24±7
	Med-IM (B)	39± 13	20 ±4	23± 11	21± 10	24±5	26±7	21±5	20±5	20±3	21±2	21±5	20±3
	Med-IP (C)	32±7	22 ±7	23±7	25±9	22±5	20±2	19± 3 E	20±2	20±2	22±4	22±2	21±3
	Med-Bup (D)	33±3	22 ±6	21±7	24± 10	24±8	18± 10	21±4	23±3	24±3	25±4	27±5	30±8
	Bup-SC (E)	33±2	23 ±8	30± 14	20±3	20±8	27±6	28± 4 C	30± 15	29± 15	25±3	29±4	28±1
Rectal temperature (°C)	Control (A)	38.8 ±0.5	37.9 ±0.4	37.8 ±0.4	37.8 ±0.3	37.5± 0.4 C,D	37.0 ±0.7	36.7± 0.6C,D	37.4 ±0.4	37.9 ±0.5	38.3 ±0.3	38.4 ±0.6	38.4 ±0.2
	Med-IM (B)	38.8 ±0.3	38.4 ±0.5	38.4 ±0.5	38.3 ±0.6	38.3 ±0.6	37.3 ±0.8	37.4 ±0.7	37.6 ±0.6	37.8 ±0.8	37.9 ±0.4	38.3 ±0.2	38.4 ±0.2
	Med-IP (C)	38.9 ±0.6	38.5 ±0.1	38.3 ±0.4	38.3 ±0.4	38.5± 0.17 A	37.5 ±0.5	37.7± 0.4 A	37.7 ±0.7	38.1 ±0.5	38.2 ±0.4	38.4 ±0.3	38.5 ±0.08
	Med-Bup (D)	38.7 ±0.5	38.6 ±0.2	38.5 ±0.2	38.5 ±0.2	38.5± 0.17 A	37.8 ±0.4	37.9± 0.2A	38.0 ±0.3	38.2 ±0.2	38.2 ±0.2	38.4 ±0.2	38.5 ±0.2
	Bup-SC (E)	38.8 ±0.4	38.1 ±0.5	38.0 ±0.4	37.9 ±0.5	37.8 ±0.6	37.3 ±0.3	37.6 ±0.3	37.6 ±0.5	37.8 ±0.6	38.0 ±0.5	38.2 ±0.4	38.2 ±0.4

Before induction of anesthesia (T0), Time of skin incision (T1), *Linea alba* incision (T2), Ligation of the first ovarian pedicle (T3), Skin suture (T4), and 30, 60, 120, 180, 240, 300, 360 minutes after the completion of surgery (T5-T11).

Table 5: Mean ± standard deviation of cardiovascular parameters in different groups at 5 minutes intervals after administration of Medetomidine

Variable	Groups	5min	10min	15min	20min
Systolic arterial pressure (mmHg)	Control (A)	*	*	*	*
	Med-IM (B)	125±13	121± 11	122± 10	110± 12
	Med-IP (C)	109±23	112± 20	107± 14	103± 10
	Med-Bup (D)	119±27	114±20	114±11	112±26
	Bup-SC (E)	*	*	*	*
Diastolic arterial pressure (mmHg)	Control (A)	*	*	*	*
	Med-IM (B)	78± 18	80± 9	73± 12	81± 12
	Med-IP (C)	69± 15	70± 12	62± 8 D	62± 9
	Med-Bup (D)	76± 11	73± 18	80± 9 C	72± 14
	Bup-SC (E)	*	*	*	*
Mean arterial pressure (mmHg)	Control (A)	*	*	*	*
	Med-IM (B)	93± 17	95± 9	89± 13	95± 13
	Med-IP (C)	79± 21	83± 12	75± 9	77± 4
	Med-Bup (D)	85± 14	89± 12	92± 6	80± 16
	Bup-SC (E)	*	*	*	*
Heart rate (beats per minute)	Control (A)	*	*	*	*
	Med-IM (B)	86±6 C,D	84± 11	87±7	75± 13
	Med-IP (C)	72± 7 B	74± 6	72± 6	69± 12
	Med-Bup (D)	69± 12 B	70± 25	71± 19	64± 20
	Bup-SC (E)	*	*	*	*
SPO_2 (%)	Control (A)	*	*	*	*
	Med-IM (B)	92.8± 5.1	93.6± 3.8	93.2± 3.2	93.4± 3
	Med-IP (C)	93.2± 3.1	94.8± 2.7	96± 4	96.4± 3.9
	Med-Bup (D)	94.2± 5.8	94.6± 6.4	94.2± 4.9	94.6± 3.3
	Bup-SC (E)	*	*	*	*

* Capital letters in each column indicate significant differences between groups ($p < 0.05$).

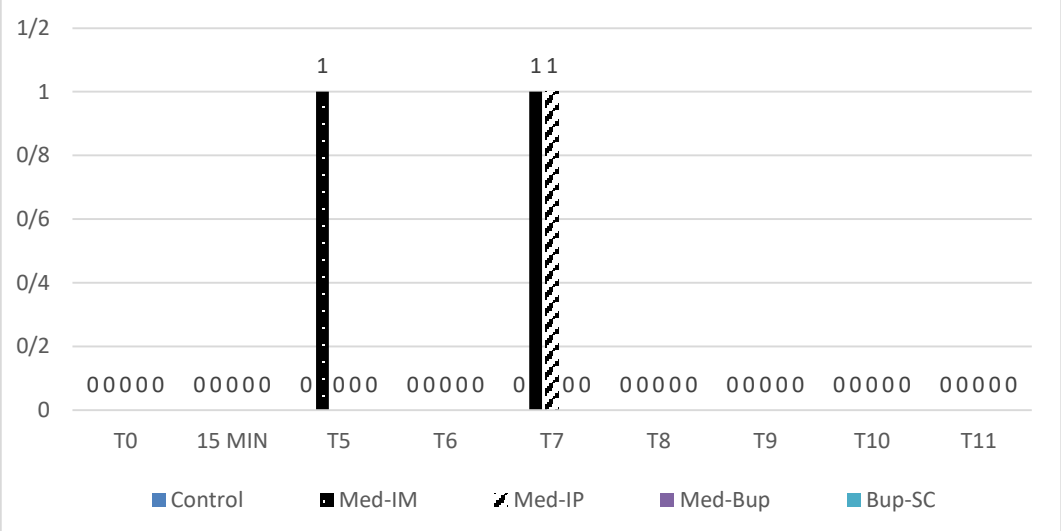

Figure 1: Median (Minimum-Maximum) Pain Assessment with SDS

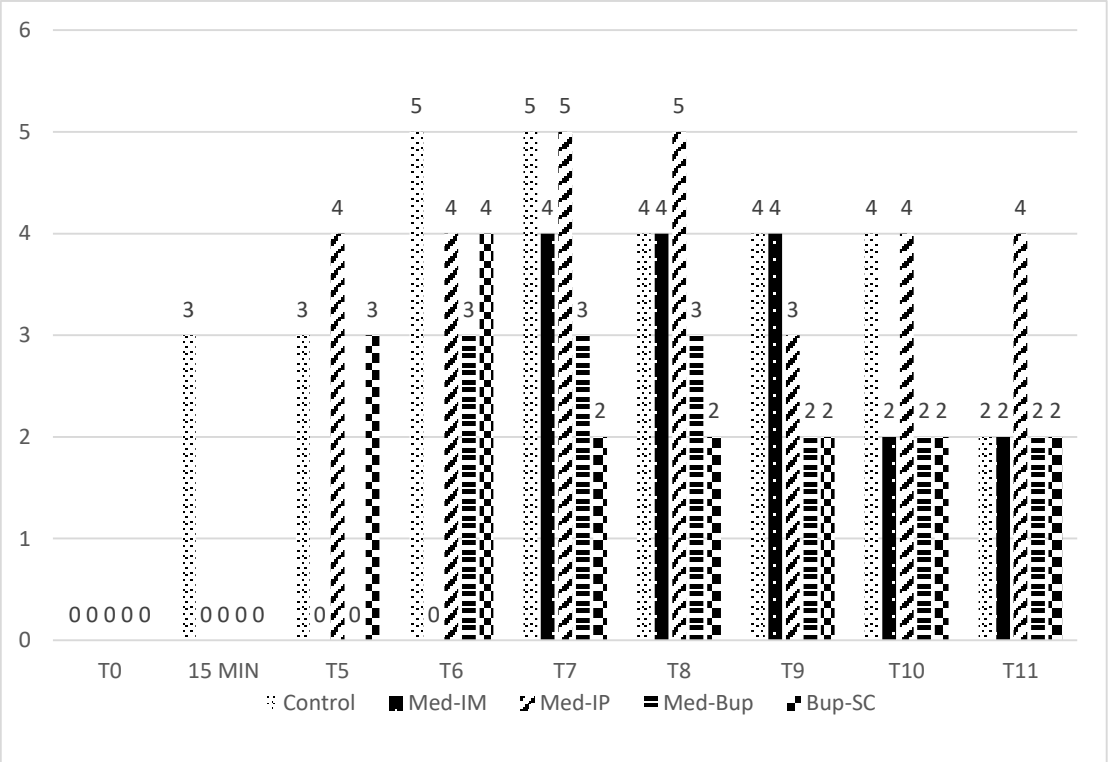

Figure 2: Median (Minimum-Maximum) Pain Assessment with VAS

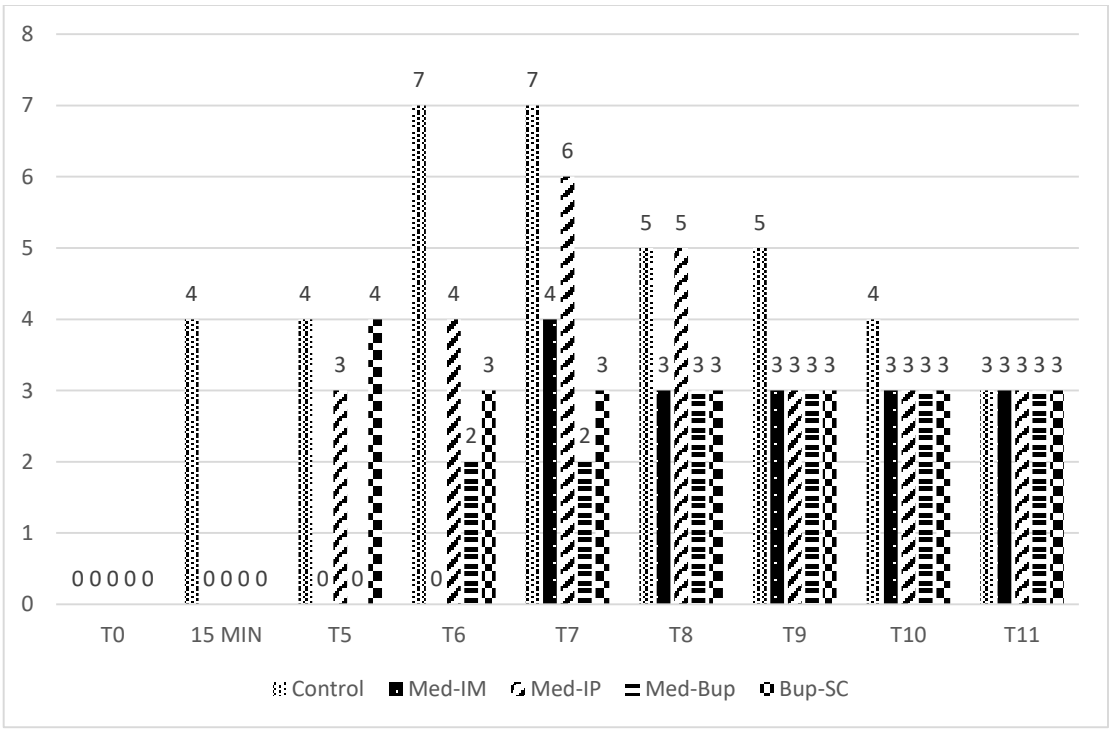

Figure 3: Median (Minimum-Maximum) Pain Assessment with UMPS

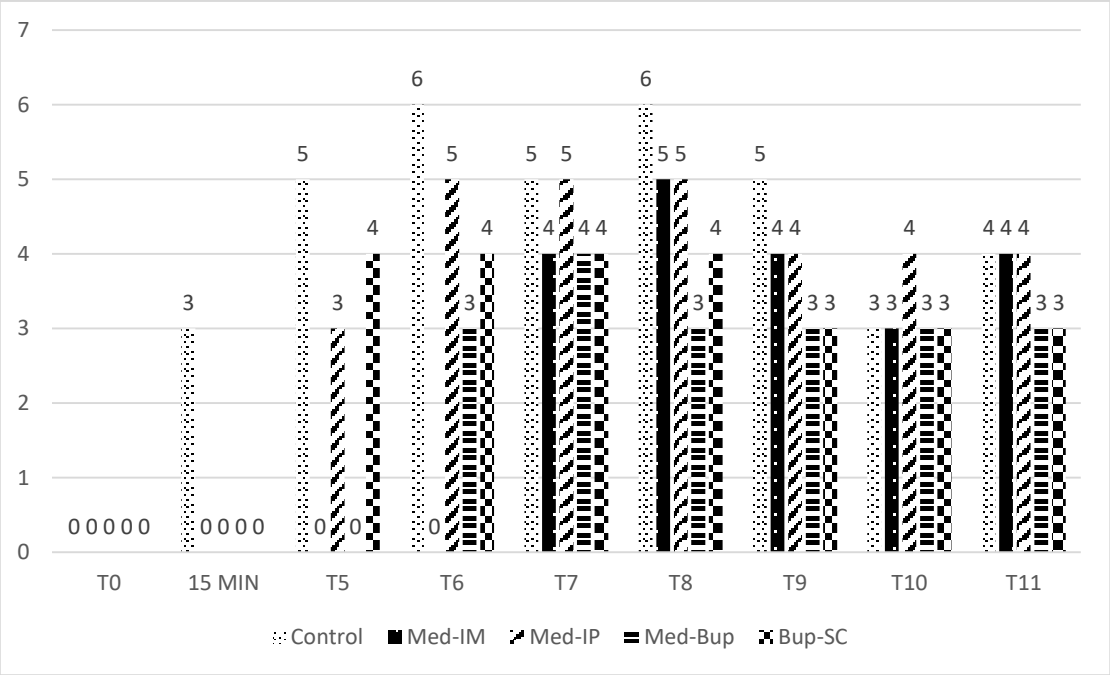

Figure 4: Median (Minimum-Maximum) Pain Assessment with CMPS-SF

Biochemical Parameters

When comparing the mean activity of lactate dehydrogenase, aspartate transaminase, and serum glucose levels in different groups, no statistically significant differences were observed among groups at different times (Table 6). However, fluctuations in the mean activity of the above enzymes and glucose levels at different times in the test groups were statistically significant.

By comparing the mean activity of CK in different groups, there was no statistically significant difference among the groups (Table 6). Furthermore, there were no statistically significant changes in the mean activity of CK at various time points in the test groups [F CK (12, 40)=1.92, P=0.06].

In the assessment of serum cortisol levels, there was a statistically significant difference among the groups. Cortisol levels in the groups that received the drug significantly decreased compared to the control group. At T1, the cortisol level in the Med-IM group increased significantly compared to the Med-Bup group ($P<0.05$). By T24, the cortisol level of the Med-IM group had increased to the same level as the control group, and this increase was significant compared to the other groups ($P<0.05$) (Table 6). The cortisol level of the Med-IM group was significantly higher than the Med-Bup in T1, and the Bup-SC group was higher than other test groups in T7. At this time, cortisol levels in all groups were lower than the control. By T24, the cortisol levels of Med-IM and control were higher than the other groups. Fluctuations in the mean serum cortisol level over time showed significant differences. In the control group, the cortisol level at T4 was lower than at other times [F cortisol (7, 37) = 2.82, P=0.017].

Table 6: Mean ± standard deviation of serum biochemical parameters in different groups at different times

Variable	Group	T0 (a)	T1 (b)	T4 (c)	T7 (d)	T11 (e)	T24 (f)
Creatine kinase (U/L)	Control (A)	130±5.5	133±13	243±9.5	293±6.5	378±11.5	244±11.5
	Med-IM (B)	133±13	180±10	220±6.7	292±10.7	337±34.6	245±7.8
	Med-IP (C)	168±10	208±8	276±8.6	309±6.5	396±23	245±38
	Med-Bup (D)	134±23	207±27	259±17.5	301±8.3	383±17.3	244±30.6
	Bup-SC (E)	172±45	222±47	262±47	337±40	412±53	249±15.7
Lactate dehydrogenase (U/L)	Control (A)	54.6±3	79±9	79.2±23	92.6±33	105.8±36	145.8±37
	Med-IM (B)	70.2±16	78.8±24	90.8±23	101.2±25	110.2±43	149.8±31
	Med-IP (C)	77.4±8	116.2±36	131±46	140±39	144.2±37	185.8±38
	Med-Bup (D)	67.2±21	84.8±25	85.4±34	109.8±42	125.8±30	139.8±24
	Bup-SC (E)	50.75±14	69.5±25	90.5±32	104.25±35	128.25±37	155.25±40
Aspartate transaminase (U/L)	Control (A)	13.2±4.9	11.51±4.7	14.43±4	15.45±3.7	20.12±4.7	18.14±8.2
	Med-IM (B)	18.12±3.5	19.34±4.3	17.37±4	19.29±5.7	24.98±5.5	18.64±3.8
	Med-IP (C)	20.34±6.9	16.87±13.1	16.76±11	25.25±12.3	30.33±7.9	22.32±6.4
	Med-Bup (D)	17.45±3.3	17±12	14.78±8.8	13.54±7.9	19.34±4.6	20.54±6.4
	Bup-SC (E)	18.65±6.9	23.33±5.5	18.76±3.1	18.34±6.1	23.43±11	20.54±10
Glucose (mg/dL)	Control (A)	94.73±10	107±38	131±62	139.79±27	112.32±23	79.29±42
	Med-IM (B)	117.7±20	134.65±31	183±97	241.51±103	132.54±43	116.36±14
	Med-IP (C)	130±24	127.27±25	129±19	164.64±51	130.3±33	113±13
	Med-Bup (D)	109.98±29	116.36±32	122.32±36	189.79±68	137.43±19	122±19
	Bup-SC (E)	111.98±32	141±51	135.65±48	135.35±41	122.2±18	116±16
Cortisol (µg/dL)	Control (A)	1.671±0.05 C,D,E,b,d, c,e,f	1.633±0.015 B,C,D,E, a,d	1.601±0.012 a,b,d,e,f	1.63±0.006 B,C,D,E,a,c,e	1.644±0.021 B,C,D,E,a,c	1.623±0.01 C,D,E,a,c
	Med-IM (B)	1.624±0.01 4 b,c,d	1.607±0.006 A,E,a,f	1.605±0.004 a,f	1.605±0.002 A,E,a,f	1.613±0.003 A	1.614±0.005 C,D,E,b,c,d
	Med-IP (C)	1.614±0.02 A,c,e,f	1.602±0.01 A	1.598±0.011 a	1.601±0.001 A,E	1.596±0.003 A,a	1.592±0.004 A,B,a
	Med-Bup (D)	1.604±0.01 7 A,b	1.589±0.009 A,a	1.594±0.002	1.601±0.001 A,E	1.602±0.004 A	1.602±0.0 A,B
	Bup-SC (E)	1.61±0.002 A	1.605±0.003 A,B	1.609±0.002	1.614±0.004 A,B,C,D,	1.608±0.003 A	1.602±0 A,B

Before induction of anesthesia (T0), Time of skin incision (T1), Skin suture (T4), 120 minutes after the completion of surgery (T7), 6 hours after surgery (T11); 24 hours after surgery (T24).

* Lowercase letters in each line indicate a significant difference between different times in each group ($p < 0.05$). ** Capital letters in each column indicate significant differences between groups ($p < 0.05$).

Discussions

We investigated the effectiveness of intraperitoneal administration of medetomidine alone and with bupivacaine at the surgical site for the pain and recovery management after ovariohysterectomy surgery in the female dogs. Pain assessment using VAS, UMPS, and CMPS-SF methods showed that medetomidine (IP) alone is not superior to intramuscular administration, in contrast to its simultaneous administration with bupivacaine, in pain management, recovery, and cardiovascular and respiratory physiological parameters.

On the other hand, administering bupivacaine alone may be more effective in reducing surgical pain and shortening recovery time compared to administering medetomidine alone or with bupivacaine. The onset time of pain reduction after surgery is faster in the bupivacaine-administered groups than in the groups that did not receive bupivacaine.

During the experiment, three dogs in the control group and two dogs in the Med-IP group received a rescue dose of morphine (0.5 mg/kg, IM) after surgery.

The anesthesia instructions in our study included the use of premedication (acepromazine and morphine), induction (propofol), and maintenance of anesthesia (isoflurane), which were administered in the same manner across all groups. These drugs, known for their sedative, anti-inflammatory, and anesthetic properties, along with the administration of medetomidine and bupivacaine, resulted in a synergistic effect in the groups studied. This synergy led to a reduction in pain scores following ovariohysterectomy in dogs. Considering the effect time and half-life of each drug during surgery and anesthesia with isoflurane, the animals experienced minimal pain and fewer side effects such as fluctuations in blood pressure, cortisol levels, glucose levels, breathing rate, and heart rate (Jiang et al, 2019).

In our study, medetomidine was administered at T3 (simultaneously with ligation of the first ovarian pedicle), and its analgesic effect was observed during the pain evaluation using CMPS-SF, VAS, and UMPS methods at T5 (half an hour post-surgery). This effect was evident until 6 hours post-surgery. Murdoch et al, (2013) investigated the analgesic effect of medetomidine. Within 10 hours post-surgery, the pain scores were significantly lower in the medetomidine group, and no animals required a rescue dose. They employed continuous infusion of medetomidine while we did not. The results in the medetomidine-IM group were consistent with Murdoch et al.'s study; but, there was a discrepancy in the medetomidine-IP group due to the differences in administration protocol (F. Murdoch et al, 2013).

The administration of morphine before ovariohysterectomy surgery to reduce pain after surgery, as outlined in our protocol, has also been supported by the previous studies. Kongara et al, (2012) suggested that giving a low dose of morphine before and tramadol after surgery had a greater impact on the duration of postoperative analgesia (Kongara et al, 2012). In a study by Karna *et al.* (2021), combining morphine (0.3 mg/kg) with dexmedetomidine or maropitant (a neurokinin-1 receptor antagonist) showed better analgesic effects compared to using morphine alone at a higher dose (0.6 mg/kg) (Karna et al, 2022). In our study, we administered morphine and acepromazine before anesthetizing female dogs to sedate them and reduce the pain during surgery. Due to the half-life of morphine, there was an analgesic effect during and after surgery.

In our study, three dogs in the control group and two dogs in the Med-IP group required a rescue dose two hours after surgery (T7). In this case, there was a significant statistical difference between the Med-IP group and the other groups (including the Med-IM group), which could be attributed to the method of medetomidine administration (Turner et al, 2011). Studies on the intraperitoneal (IP) administration method and its limitations have shown that one limitation is first-pass metabolism, similar to what is observed with orally administered medications because drugs absorbed from the peritoneal cavity end up in the portal vein and pass through the liver. Therefore, the pharmacokinetics of drugs administered IP are similar to oral administration in terms of metabolic fate and the first-pass metabolism, resulting in lower bioavailability compared to intramuscular or intravenous administration. Additionally, the time to reach the maximum plasma concentration (Cmax) with IP administration is longer than with IM administration (Al Shoyaib et al, 2019). As a result, the pain reduction time with IM medetomidine was faster than with the IP method. The difference in Cmax levels between the two administration methods contributed to more pain reduction with the IM method compared to the IP method.

In a study by Saponaro et al, (2013), the cardiovascular effects of medetomidine (2

µg/kg), acepromazine (20 µg/kg), and their combination were investigated intravenously in healthy dogs. The blood pressure and non-invasive echocardiography were measured at 0, 15, 50, and 80 minutes after drug administration. The results showed a decrease in the left ventricular afterload due to the acepromazine and an increase in the right ventricular afterload caused by medetomidine. However, the combination of these drugs reduced the mentioned effects and prevented the occurrence of atrioventricular block (Saponaro et al, 2013).

Our study focused on examining the physiological parameters of cardiovascular, respiratory, and rectal temperature. The protocol applied before, during, and after surgery successfully maintained these parameters within the physiological range.

One known side effect of medetomidine is a transient increase in blood pressure followed by a decrease (Sinclair, 2003). The timing of medetomidine administration may explain the significant increase in blood pressure at T4. Additionally, in the group that received intraperitoneal medetomidine, the time to reach Cmax and different bioavailability prevented similar effects seen with IM administration (Al Shoyaib et al, 2019).

The study by Ramesha et al, (2022) compared the pain management, quality of recovery, and physiological parameters during and after surgery using a combination of medetomidine and dexmedetomidine with propofol as an anesthesia inducer, versus using propofol alone without medetomidine or dexmedetomidine in dogs. Their results showed that in the groups where propofol was combined with one of these drugs, the anesthesia induction was faster, physiological parameters remained within normal range, and sedation, analgesia, and quality of recovery were not associated with adverse effects (Ramesha et al, 2022). In our study, propofol was an induction agent and sedation achieved in all groups when combined with medetomidine. When examining the quality of recovery, groups that received medetomidine scored higher degree than the other groups (i.e. poorer recovery quality with more ataxia and struggling to stand). One of the side effects of using medetomidine is its impact on blood flow to motor muscles, caused by an increase in deoxygenated hemoglobin concentration. This effect, combined with the decrease in blood pressure and bradycardia induced by the drug, can affect the animal's recovery time (Sinclair, 2003).

Bupivacaine is an aminoamide that blocks the action potential in nerve cells by blocking sodium channels and is used as a local anesthetic (Shafiei et al, 2018). In a prospective randomized clinical study, Campagnol et al, (2012) compared the effect of intraperitoneal or incisional bupivacaine on pain and the need to rescue dose after ovariohysterectomy in dogs. They suggested that intraperitoneal bupivacaine led to lower pain scores in the first hour after surgery and there was a trend towards reducing the need for rescue analgesia after ovariohysterectomy in dogs (Campagnol et al, 2012). Results of their study were consistent with the results of ours in terms of pain reduction and no need for a rescue dose in the groups receiving bupivacaine. In the study by Shankar *et al.* (2022), the analgesic effect of bupivacaine alone and in combination with dexmedetomidine was investigated in laparoscopic surgery. They assessed the quality of analgesia and the time to first request for a rescue dose. Their findings indicated that the co-administration of dexmedetomidine (1 µg/kg; IP) with bupivacaine (0.25%) resulted in decreased post-operative pain and reduced the need for a rescue dose compared to bupivacaine alone (Shankar et al., 2022). The results of these studies consistently demonstrated that combining bupivacaine with a α2 agonist leads to pain relief and eliminates the need for a rescue dose.

In the present study, there was no significant difference in blood glucose levels between different groups; however, the glucose levels fluctuated in the groups over time. At T7 (two hours after surgery), the highest level of glucose was observed in the groups receiving medetomidine, followed by the group that received only bupivacaine. In stress and pain conditions, cortisol levels elevate, and its effect on gluconeogenesis leads to increased serum glucose levels. The higher the cortisol level, the higher the glucose level (Hannibal and Bishop, 2014). The results of our study showed a correlation between glucose and cortisol fluctuations over time, but the cortisol levels in all test groups had decreased compared to the control group. One potential reason for the increased glucose levels in the medetomidine groups compared to other test groups is the effect of medetomidine as an α2 agonist on the pancreatic islets. This results in the suppression of insulin secretion in response to sympathetic stimulation, leading to increased blood sugar (Hampton et al, 2022).

Due to the liver metabolism and cardiac effects of the medicine used in the protocol of this study, biochemical parameters associated with heart and liver function were investigated to determine the adverse effects of the doses used. The activity of lactate dehydrogenase, and aspartate aminotransferase were all within the normal range, and no significant differences were observed in the test groups. The activity of the creatine kinase enzyme was within the normal range in all groups. Creatine kinase activity increases in conditions such as coronary artery bypass surgery, heart transplantation, myocarditis, and pulmonary embolism after surgery. Creatine kinase activity rises within 12 hours after muscle injury, peaks at 24 to 36 hours, and returns to normal after 48 to 72 hours (Aujla and Patel, 2019). In our study, the highest increase in the activity of this enzyme was observed 8 hours after surgery (T11) in all groups, which was likely caused by the surgical incision.

There were limitations in our study. One limitation was in assessing the pain after surgery using various tests. We applied a multimodal analgesia approach in all groups; so, the results of pain assessment could not definitively be attributed to our study target drugs (medetomidine and bupivacaine) due to the synergistic impact of these drugs. Morphine was used in our protocol as a sedative and pre-emptive analgesia, with duration of action of about 3-8 hours. According to our procedure, its effect may last at least 7 hours after surgery (T10) and affect the pain evaluation results. Additionally, the combination of acepromazine, morphine, propofol, and isoflurane reduces the blood pressure, which can affect the effect of target drugs in this study on blood pressure during and after surgery (depending on the half-life).

We compared IM and IP medetomidine administration methods in dogs undergoing ovariohysterectomy surgery. Due to differences in bioavailability, Cmax, and time to reach Cmax between the two administration methods, using the same dose of medetomidine can impact the conclusion of which administration method is preferable. The future research should consider this issue and use appropriate doses for each administration method.

The sample size in this study was small, although it was sufficient to achieve our study goals regarding the effect of medetomidine and bupivacaine in inducing analgesia during and after the surgery. However, a larger sample size is needed to draw more definitive conclusions about the analgesic and sedation effects of medetomidine. Despite the multimodal analgesia approach, consistent analgesia was not observed in all animals, underscoring the importance of individualized pain assessment.

When evaluating the pain using VAS, UMPS, and CMPS-SF methods, there was no evidence to suggest that the effectiveness

of administering the same dose of intraperitoneal medetomidine alone was superior to intramuscular administration of medetomidine alone or in combination with bupivacaine at the surgical site for pain management, quality of recovery after Ovariohysterectomy surgery, and cardiovascular parameters in the female dogs.

Conversely, administering bupivacaine alone significantly reduced surgical pain and recovery time compared to administering medetomidine alone or in combination with bupivacaine. Animals receiving intraperitoneal medetomidine required a rescue dose, while no rescue dose was needed in the other groups. Doses of all medications used in each group did not interfere with the animals' physiological functions, and cardiovascular, respiratory, and rectal temperature results remained within normal ranges. Additionally, levels of serum enzymes related to liver and heart tissue function stayed within normal ranges.

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Conflict of Interest

The authors declare that they have no known conflict of interest.

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References

Abdulkhaleq, L. A., Assi, M. A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y. H., & Hezmee, M. N. M. (2018). The crucial roles of inflammatory mediators in inflammation: A review. *Vet World, 11*(5), 627-635. doi: 10.14202/vetworld.2018.627-635

Al Shoyaib, A., Archie, S. R., & Karamyan, V. T. (2019). Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? *Pharmaceutical Research, 37*(1), 12. doi: 10.1007/s11095-019-2745-x

Aujla, R. S., & Patel, R. (2019, 2024 Feb 27). Creatine phosphokinase. Retrieved 2024 Jan, from https://www.ncbi.nlm.nih.gov/books/NBK546624/

Baniadam, A., MASOUMI, p., Ezzati Givi, M., & Razi Jalali, M. (2021). Comparison of analgesic and cardiopulmonary effects in epidural injection of lidocaine, bupivacaine and dexmedetomidine following ovariohysterectomy in the dog. *Iranian Veterinary Journal, 17*(3), 14-23. doi: 10.22055/ivj.2021.253465.2313

Bencharif, D., Amirat, L., Garand, A., & Tainturier, D. (2010). Ovariohysterectomy in the Bitch. *Obstetrics and Gynecology International, 2010*, 542693. doi: 10.1155/2010/542693

Bussen, S., Sütterlin, M., & Steck, T. (1999). Endocrine abnormalities during the follicular phase in women with recurrent spontaneous abortion. *Hum Reprod, 14*(1), 18-20. doi: 10.1093/humrep/14.1.18

Campagnol, D., Teixeira-Neto, F. J., Monteiro, E. R., Restitutti, F., & Minto, B. W. (2012). Effect of intraperitoneal or incisional bupivacaine on pain and the analgesic requirement after ovariohysterectomy in dogs. *Vet Anaesth Analg, 39*(4), 426-430. doi: 10.1111/j.1467-2995.2012.00728.x

Chilkoti, G. T., Kumar, M., Mohta, M., Saxena, A. K., Sharma, N., & Singh, J. (2019). Comparison of postoperative analgesic efficacy of low-dose bolus intravenous dexmedetomidine and intraperitoneal dexmedetomidine with bupivacaine in patients undergoing laparoscopic cholecystectomy: A randomised, controlled trial. *Indian J Anaesth, 63*(2), 106-113. doi: 10.4103/ija.IJA_440_18

Hampton, R. F., Jimenez-Gonzalez, M., & Stanley, S. A. (2022). Unravelling innervation of pancreatic islets. *Diabetologia, 65*(7), 1069-1084. doi: 10.1007/s00125-022-05691-9

Hannibal, K. E., & Bishop, M. D. (2014). Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys Ther*, *94*(12), 1816-1825. doi: 10.2522/ptj.20130597

Jiang, M., Mieronkoski, R., Syrjälä, E., Anzanpour, A., Terävä, V., Rahmani, A. M., . . . Liljeberg, P. (2019). Acute pain intensity monitoring with the classification of multiple physiological parameters. *Journal of clinical monitoring and computing*, *33*, 493-507.

Kamohara, H., Kamohara, T., & Hikasa, Y. (2022). A randomized clinical trial on effects of alfaxalone combined with medetomidine and midazolam in preventing stress-related neurohormonal and metabolic responses of isoflurane-anesthetized cats undergoing surgery. *Am J Vet Res*, *83*(11), 1-10. doi: 10.2460/ajvr.22.03.0041

Kandi, S., Panigrahy, L. K., Dhamudia, H. C., Patel, P. K., & Sethi, P. (2022). Efficacy of intravenous dexmedetomidine versus intraperitoneal dexmedetomidine in laparoscopic cholecystectomy–A prospective randomized double-blinded study. *Asian Journal of Medical Sciences*, *13*(8).

Kania, B. F., Wrońska, D., & Bracha, U. (2021). Pain, Pathophysiological Mechanisms, and New Therapeutic Options for Alternative Analgesic Agents in Sheep: A Review and Investigation. *Animals (Basel)*, *11*(3). doi: 10.3390/ani11030909

Karna, S., Chambers, P., Singh, P., Lopez-Villalobos, N., & Kongara, K. (2022). Evaluation of analgesic interaction between morphine, maropitant and dexmedetomidine in dogs undergoing ovariohysterectomy. *New Zealand Veterinary Journal*, *70*(1), 10-21.

Kongara, K., Chambers, J., & Johnson, C. (2012). Effects of tramadol, morphine or their combination in dogs undergoing ovariohysterectomy on peri-operative electroencephalographic responses and post-operative pain. *New Zealand Veterinary Journal*, *60*(2), 129-135.

Lambertini, C., Kluge, K., Lanza-Perea, M., Bruhl-Day, R., & Kalchofner Guerrero, K. S. (2018). Comparison of intraperitoneal ropivacaine and bupivacaine for postoperative analgesia in dogs undergoing ovariohysterectomy. *Vet Anaesth Analg*, *45*(6), 865-870. doi: 10.1016/j.vaa.2018.06.012

Mair, A. R., Pawson, P., Courcier, E., & Flaherty, D. (2009). A comparison of the effects of two different doses of ketamine used for co-induction of anaesthesia with a target-controlled infusion of propofol in dogs. *Vet Anaesth Analg*, *36*(6), 532-538. doi: 10.1111/j.1467-2995.2009.00500.x

Muir, W., Lerche, P., Wiese, A., Nelson, L., Pasloske, K., & Whittem, T. (2009). The cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in cats. *Vet Anaesth Analg*, *36*(1), 42-54. doi: 10.1111/j.1467-2995.2008.00428.x

Murdoch, F., Maker, G., Nitsos, I., Polglase, G., & Musk, G. C. (2013). Intraperitoneal medetomidine: a novel analgesic strategy for postoperative pain management in pregnant sheep. *Laboratory Animals*, *47*(1), 66-70.

Murdoch, F. R., Maker, G. L., Nitsos, I., Polglase, G. R., & Musk, G. C. (2013). Intraperitoneal medetomidine: a novel analgesic strategy for postoperative pain management in pregnant sheep. *Lab Anim*, *47*(1), 66-70. doi: 10.1177/0023677212473712

Raekallio, M. R., Virtanen, M., Happonen, I., & Vainio, O. M. (2017). Adverse reactions of α2-adrenoceptor agonists in cats reported in 2003–2013 in Finland. *Veterinary Anaesthesia and Analgesia*, *44*(4), 803-810. doi: https://doi.org/10.1016/j.vaa.2016.07.008

Ramesha, H., Mahesh, V., Nagaraja, B., Sudha, G., Ranganath, L., & Srinivasa Murthy, K. (2022). *Comparative studies on medetomidine and dexmedetomidine as pre-anaesthetics for propofol-isoflurane general anesthesia in dogs.* (PhD PhD dissertation), Karnataka Veterinary, Animal and Fisheries Sciences University India.

Rezaeipour, A., Naddaf, H., Jalali, S. M., & Sabiza, S. (2022). Evaluation of intraperitoneal administration of morphine on post-operative pain management after ovariohysterectomy in dogs. *Vet Med Sci*, *8*(1), 150-156. doi: 10.1002/vms3.668

Saponaro, V., Crovace, A., De Marzo, L., Centonze, P., & Staffieri, F. (2013). Echocardiographic evaluation of the cardiovascular effects of medetomidine, acepromazine and their combination in healthy dogs. *Research in veterinary science*, *95*(2), 687-692.

Shafiei, F. T., McAllister, R. K., & Lopez, J. (2018, 2023 Aug 17). Bupivacaine. Retrieved 2024 Jan, from https://www.ncbi.nlm.nih.gov/books/NBK532883/

Shankar, S., Gupta, B. K., Singh, M. K., Pandey, A. R., Dwivedi, V., & Sachan, S. (2022). Intraperitoneal bupivacaine alone or with dexmedetomidine for post-operative analgesia following laparoscopic cholecystectomy: a prospective randomized comparative study. *Anaesthesia, Pain & Intensive Care, 26*(3), 347-351.

Sinclair, M. D. (2003). A review of the physiological effects of α2-agonists related to the clinical use of medetomidine in small animal practice. *The Canadian veterinary journal, 44*(11), 885.

Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science, 50*(5), 600-613.

Walters, E. T. (2018). Defining pain and painful sentience in animals. *Animal Sentience, 3*(21), 14.

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مقایسه تجویز داخل صفاقی مدتومیدین و پیرامون برش بوپیواکائین بر کنترل درد بعد از جراحی اواریوهیسترکتومی در سگ

سید محمد سجادی[۱]، علی بنی‌آدم[۲]، سروش سابیزا[۲*] و سیده میثاق جلالی[۲]

[۱] دانشجوی دکتری تخصصی جراحی دامپزشکی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۲] دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

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چکیده

ایجاد شرایط بی‌دردی مؤثر بعد از اعمال جراحی در دامپزشکی به خصوص متعاقب اعمالی چون عقیم‌سازی حیوانات امری بسیار مهم از نظر اخلاقی و مراقبت بالینی به شمار می‌آید. به منظور دستیابی به این هدف از داروهای ضد درد گوناگونی تا به امروز استفاده شده است، اما در حیوانات مختلف مدیریت درد حین و بعد از جراحی نیازمند استراتژی‌های متفاوتی است. در این مطالعه، آثار دو داروی مدتومیدین و بوپیواکائین در اشکال متفاوت تجویز بر روی کنترل درد بعد از عمل جراحی اواریوهیسترکتومی بر روی سگ‌های ماده مورد بررسی قرار گرفت. ۲۵ سگ ماده نژاد بومی (۱-۴ سال سن و ۱۵-۲۵ کیلوگرم وزن) بر اساس نوع دارو و روش تجویز به ۵ گروه ۵ تایی تقسیم شدند: گروه کنترل، گروه مدتومیدین عضلانی (Med-IM)، گروه مدتومیدین صفاقی (Med-IP)، گروه مدتومیدین صفاقی همراه با بوپیواکائین زیر جلدی اطراف برش (Med/Bup) و گروه بوپیواکائین زیرجلدی اطراف برش (Bup-Sc). در تمامی گروه‌ها داروها در سه نقطه زمانی تجویز شد؛ قبل از برش پوست، همزمان با لیگاتور اولین پایه تخمدان و پیش از بستن برش خط وسط شکم. در زمان‌های قبل از جراحی، حین و بعد از اتمام عمل جراحی کیفیت آرامبخشی، کیفیت درد، عمق بیهوشی، کیفیت ریکاوری، علائم حیاتی (دمای بدن، تعداد تنفس، شاخص‌های قلبی عروقی) و شاخص‌های هماتولوژیک، بیوشیمیایی اندازه‌گیری شد. امتیاز درد بعد از جراحی با کمک روش‌های امتیاز توصیفی ساده (SDS)، مقیاس بصری آنالوگ (VAS)، مقیاس درد ملبورن (UMPS) و فرم کوتاه مقیاس درد گلاسگو (CMPS-SF) مورد ارزیابی قرار گرفت. داده‌های به دست آمده با استفاده از نرم‌افزار SPSS و به کارگیری آزمون‌های مناسب مورد ارزیابی و تحلیل قرارگرفت. نتایج این مطالعه نشان داد که تجویز داخل صفاقی داروی مدتومیدین با دوز مشابه تزریق عضلانی این دارو منجر به اختلافات معنی‌داری در امتیاز درد (بر اساس روش‌های CMPS-SF و VAS)، ضربان قلب و سطوح سرمی کورتیزول در زمان‌های به خصوص بعد از عمل جراحی شد. تجویز زیرجلدی اطراف برش بوپیواکائین به تنهایی در مقایسه با تجویز مدتومیدین به تنهایی و یا مدتومیدین و بوپیواکائین همراه با یکدیگر، به طرز معنی‌داری باعث کاهش امتیاز درد بعد از عمل جراحی و کاهش مدت زمان ریکاوری شد. در گروه مدتومیدین صفاقی، حیوانات نیازمند دریافت دوز ضددرد نجات دهنده شدند در حالی که در سایر گروه‌های تیمار این نیاز ایجاد نگردید. مقدار داروهای تجویز شده در این مطالعه اختلالی در عملکردهای فیزیولوژیک هیچ یک از سگ‌ها ایجاد نکرد و شاخص‌های قلبی عروقی، دستگاه تنفس و دمای مقعدی در محدوده طبیعی خود باقی بود. فعالیت سرمی آنزیم‌های کبدی و عملکرد قلبی نیز در محدوده طبیعی خود بود. ارزیابی امتیاز درد با استفاده از روش‌های VAS، UMPS و CMPS-SF هیچ گونه ارجحیت یا برتری در تجویز داخل صفاقی داروی مدتومیدین نسبت به تجویز عضلانی آن (با دوز مشابه) یا تجویز عضلانی همین دارو همراه با بوپیواکائین در کنترل درد بعد از جراحی عقیم سازی سگ ماده و ریکاوری بعد از جراحی، پارامترهای فیزیولوژیک، قلبی عروقی، تنفسی و دمای مقعدی نشان نداد.

کلمات کلیدی: مدیریت درد، اواریوهیسترکتومی، سگ ماده، مدتومیدین، بوپیواکائین

* نویسنده مسئول: سروش سابیزا، دانشیار، گروه علوم درمانگاهی، دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: s.sabiza@scu.ac.ir