Original Article

Comparison of the postoperative outcome of the three-port laparoscopic ovariectomy and conventional open ovariectomy methods in dogs

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Abstract

The aim of this study is to determine the effects of laparoscopic ovariectomy (LOVE) and conventional open ovariectomy (COVE) methods on early-stage surgical stress parameters [proinflammatory cytokines (TNF-α and IL-6), acute phase proteins (CRP, Hp), inducible nitric oxide synthase (INOS), mitogen activated protein kinase (MAPK14) and cortisol], pain scores and D-Dimer (DD) levels in 36 healthy bitches. Three-port laparoscopic ovariectomy was performed on the LOVE group while routine ovariectomy was performed on the COVE group. Surgical stress parameters and pain scores (University of Melbourne pain scale) were assessed during the pre- (0 hour) and postoperative periods (1st, 3rd, 6th hour and 7 days). DD analyses were performed between 0-1 hours to compare coagulation rates. At the early postoperative stage, operation time, surgical stress, pain scores and DD levels were lower (P<0.05) in the LOVE group compared to the COVE group. No differences were found in terms of surgical stress and pain score between the groups on day 7 post-operation. As a result, we found that LOVE, a minimal invasive method, caused less surgical stress and post-operative pain as well as a shorter operation period compared to COVE and the patients returned to their preoperative activities in a shorter time. Therefore, it was concluded that the three-port LOVE was more advantageous compared to COVE in terms of safety and patient comfort.

Keywords: Acute phase response, bitches, D-Dimer, three-port laparoscopy, ovariectomy, pain score

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Introduction

Ovariectomy (OV) and ovariohysterectomy are the two most commonly used techniques for the sterilization of female small animals (Katić and Dupré, 2017). Today the tendency towards using minimal invasive techniques in small animal surgery has increased and laparoscopic OV has become on the most common clinical practices in the veterinary field (Katić and Dupré, 2017). Despite the fact that conventional open surgical methods have advantages, such as fast manipulation, easy performance and the ability to view the operation area in 3 dimensions, it may lead to an increase in surgical stress (Matsumoto *et al*., 2005). Although the learning curve and cost of laparoscopic procedures are discouraging at first, they are reported to have several advantages over the conventional open surgical techniques, such as a short incision line and better vizualization of the abdominal cavity (Devitt *et al*., 2005; Haraguchi *et al*., 2017; Lansdowne *et al*., 2012). For this reason, laparoscopic surgery is becoming a method that is increasingly preferred by veterinarians and requested by owners (Gower and Mayhew, 2008).

Nowadays, it is generally accepted that surgical procedures have certain risk factors, in terms of hypercoagulation and thrombosis (Rosendaal, 1999). DD is a degradation product cross-linked fibrin which can increase with clot formation and fibrinolysis (Epstein *et al*., 2013). It has been reported in dogs that the concentration of DD increases in the postoperative period due to thromboembolism and internal bleeding (Epstein *et al*., 2013).

Surgical stress is a general definition that includes hormonal, metabolic and inflammatory reactions that occur in the body due to surgical trauma (Tvarijonaviciute *et al*., 2015). Ovariectomy is a model that is frequently used in veterinary medicine to assess the surgical stress and inflammation (Del Romero *et al*., 2020). Acute phase response (APR) and cortisol are the parameters that are used to mirror the stress induced by surgery (Jacobsen *et al*., 2009). INOS (Kuyumcu *et al*., 2004) and MAPK14 (Schäfer and Williams, 2000) are also used today as stress indicators.

The aim of this study was to determine the effects of surgical techniques LOVE and COVE on the surgical stress parameters, postoperative pain scores, and DD levels of dogs post-operation.

Materials and Methods

Animals: Thirty-six entirely healthy bitches of different breeds were initially included. The dogs were between 7 and 40 months of age and weighed between 18 and 37 kg. The study was approved by the Local Ethics Committee for Animal Experiments of the University of Atatürk (2019/E.1900373444). All animal owners signed a consent form after the relevant project information was explained to them. Prior to surgery, dogs underwent a thorough clinical examination to ensure that they were completely healthy. Complete blood cell count and biochemistry profile, physical, ultrasonographic examination and vaginal cytology were performed. Bitches determined to be healthy were included in the study groups.

Study Groups: The thirty-six bitches were randomly divided into two groups, according to the surgical technique: 18 animals were allocated to the group LOVE and 18 animals were allocated to the group COVE.

Anesthesia and Antibiotic Prophylaxis: Both groups were administered medetomidine hydrochloride (0,04 mg/kg, Domitor®, Zoetis, Turkey) by intramuscular (IM) route for premedication. Propofol (2-3 mg/kg, Propofol-Lipuro®, Braun, Germany) was administered by intravenous (IV) route for induction following moderate sedation. General anaesthesia was maintained throughout the operation using isoflurane (1%, Isoflurane, Piramal Critical Care Inc., USA) in 50% O_2. All dogs were administered Lactated Ringer's Solution (5 ml/kg/hour, IV, Polifarma İlaç A.Ş., Turkey) throughout the operation. A single dose of Sefazol (20 mg/kg, IM, Iespor, I.E. Ulagay İlaç Sanayi, Turkey) was administered during the preoperative period. During the operations, respiratory rate, heart rate, ECG and O_2 saturation were monitored.

Surgical Procedures: Two surgical techniques for ovariectomy were applied, namely the LOVE and COVE approaches. All techniques were performed by the same surgeon with the support of an assistant. The surgical area was clipped and scrubbed. COVE was performed using a standard open surgical technique (Kowaleski, Boudrieau *et al*. 2012). For the group LOVE, a three-cannula laparoscopic technique (Katić and Dupré 2017) was used. The caudal portal is set halfway between the umbilicus and the *os pubis*; this portal is usually used for the telescope (5 usually 0°). The middle portal is set at 1 cm caudal to the umbilicus and the cranial portal is set at approximately 1 cm cranial to the umbilicus. Cannulas used were the same size (5 mm). Pneumoperitoneum was established by insufflating CO_2 to a pressure of 8-12 mm Hg. The patient was rotated into right lateral oblique recumbency for removing the left ovary and subsequently into left lateral oblique recumbency for removing the right ovary. Ovaries were removed through the cranial 5-mm cannula after sealing the ovarian vessels using the LigaSure™ (Blunt Tip, Covidien, Medtronic, Turkey) sealing device. The time of surgery was recorded for each dog and was defined as the time from the start of the skin incision to the time of last suture placement. Surgery duration was calculated and compared between techniques.

Blood Sampling and D-Dimer Analysis: Before both surgical procedures (before anesthesia; 0 hour) and at 1, 3, 6 hours and on day 7 post-operation, blood samples of 5 ml were taken from cephalic vein and kept at room temperature for 30 minutes and centrifuged (3000 rpm, 10 min.) afterwards. The serums were stored in Eppendorf microtubes at -80 °C until they were analyzed. Blood samples of 2 ml were taken into 3.2% sodium citrate tubes pre- and post-operation for DD analyses. Plasma DD level was analyzed using the Genrui FA50.

University of Melbourne Pain Scale (UMPS): The bitches were assessed by the same handler who was unaware of which procedure had been performed. The more painful behaviors were weighted with increasingly higher scores. At designated times (before anesthesia and 1, 3, 6 hours and 7 days post-operation), the handler assessed each dog through a closed window to avoid disturbing the patient. After observations were recorded, physiological variables were assessed for each dog.

Post-Operative Management and Sampling for Stress Parameters: Following the surgery, the dogs were administered meloxicam (0.2 mg/kg SC). Each dog had an identical light abdominal bandage applied so the handler was unaware of each dog's specific surgical procedure post-operation. If at any time during the study the dogs had UMPS scores >10, meloxicam (0.22 mg/kg) was administered subcutaneously. Overall status, incision line, clinical recovery and complications of all the dogs were routinely checked on a daily basis. On day 7 of wound recovery, APR, INOS, MAPK14 and pain score measurements were repeated to assess the return of the dogs to their pre-operative activities.

Statistical Analysis: All data were analyzed the using SPSS 22.0 (IBM company, version 22, SPSS Inc, USA). Data were checked through Shapiro-Wilk test for normal distrubition. Normally distributed data were analyzed by One-way ANOVA with repeated measures (effect of group, time, and group by time interaction). Differences pre-and post-treatment values of DD within the groups were compared with a paired t-test. The TNF-α-INOS, and TNF-α-MAPK14 were calculated by Pearson's correlation coefficient. Data are shown as mean ± standard error (SE). A P-value of < 0.05 was considered statistically significant.

Results

No statistical differences were observed between the dogs in the LOVE and COVE groups in terms of age (7-40; 15.7 ± 12.1; P=0.37) or weight (18-37 kg; 13.7 ± 9.4; P=0.64).

There were differences (*P*<0.05) in the LOVE (18.6 ± 2.01 min) and COVE (33.2 ± 2.33 min) groups in terms of average operation duration.

No difference was found between the groups in terms of UMPS pain score as expected, in the pre-operative period (0 hour). The LOVE group had lower pain scores at 1, 3 and 6 hours post-operation compared to the COVE group (*P*<0.05). No difference was found between the groups on day 7 post-operation (Figure 1).

No difference was found between the groups in terms of cortisol (Figure 2), TNF-α (Figure 3), IL-6 (Figure 4), CRP (Figure 5), Hp (Figure 6), INOS (Figure 7) and MAPK14 (Figure 8) levels during the pre-operative period (0 hour). However, all levels were higher for the dogs in the COVE group compared to the LOVE group at 1-, 3- and 6-hours post-operation. No difference was found between the groups on day 7 post-operation (Table 1).

No statistical difference was found between the preoperative (0 hour) DD levels of the dogs in both groups. It was found that the DD levels in the postoperative period were lower in the LOVE group (0.16±0.25) compared to the COVE group (0.22±0.25) (*P*<0.05) (Figure 9).

There was a positive correlation between TNF-α and MAPK14 (r = 0.922, P = 0.026) and TNF-α and INOS (r = 0.820, P = 0.089).

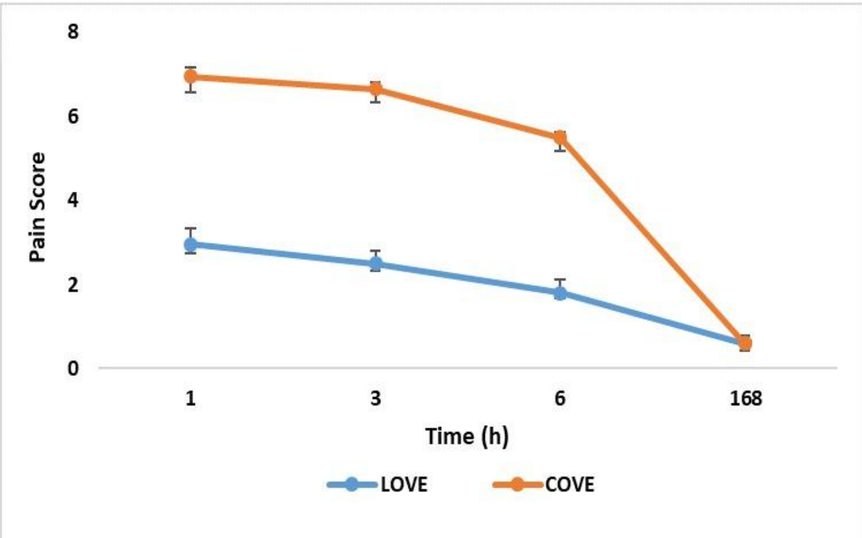

Figure 1 The serum UMPS (pain score) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

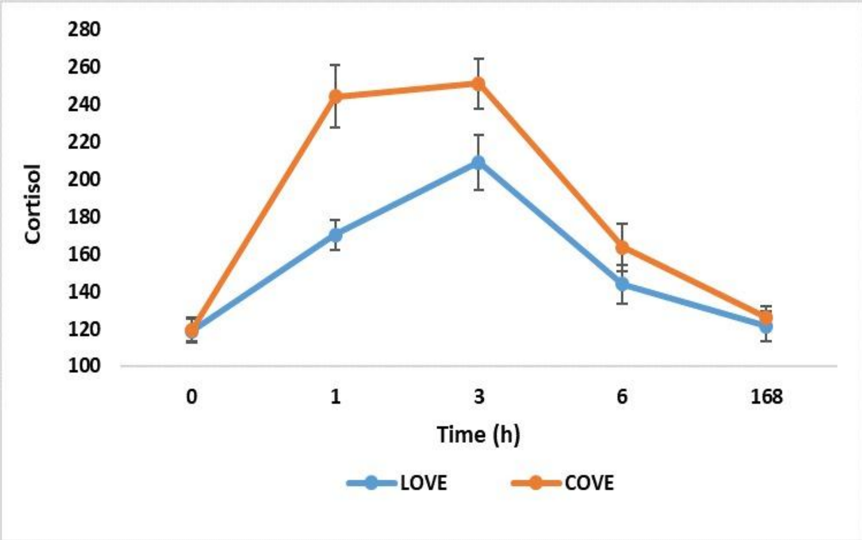

Figure 2 Comparison of serum cortisol concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

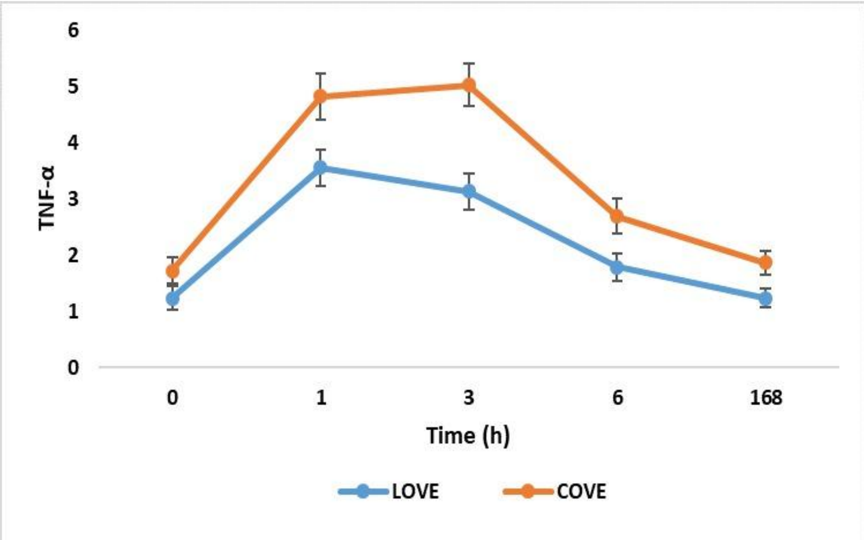

Figure 3 Comparison of serum tumor necrosis factor alpha (TNF-α) laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

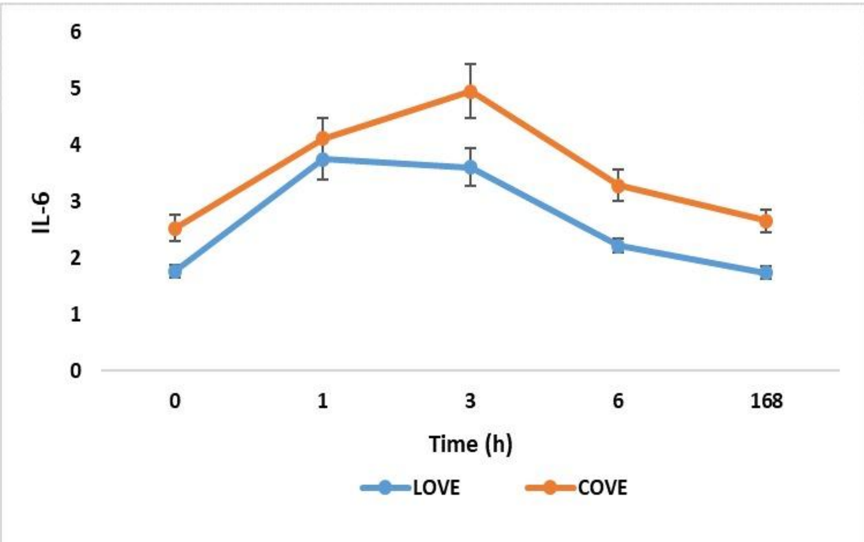

Figure 4 Comparison of serum interleukin-6 (IL-6) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

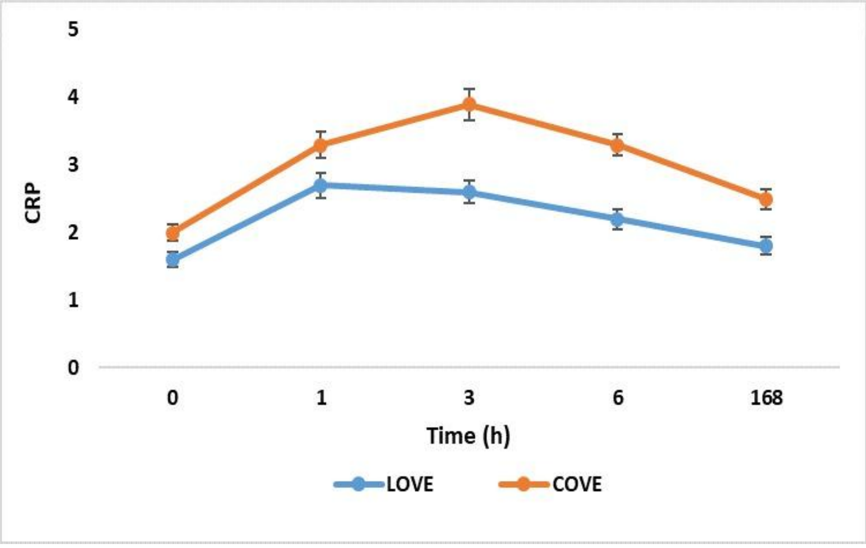

Figure 5 Comparison of serum C-reactive protein (CRP) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

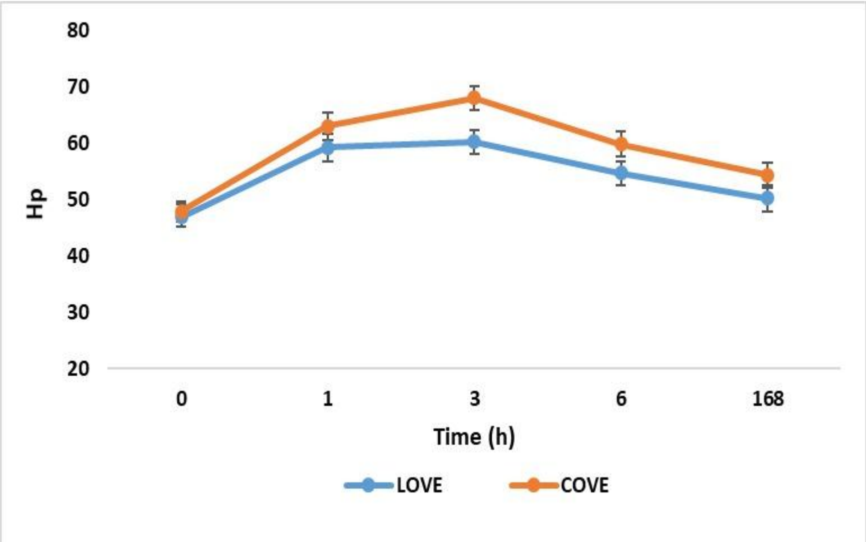

Figure 6 Comparison of serum haptoglobin (Hp) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

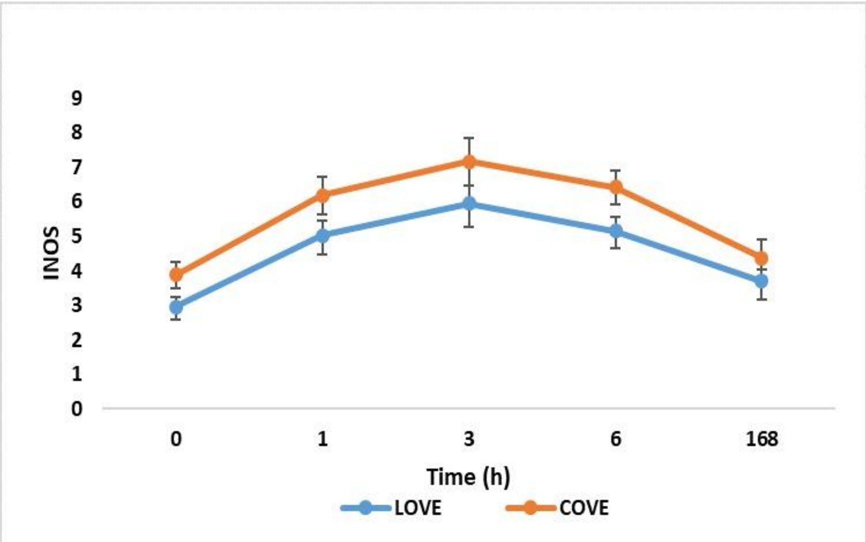

Figure 7 Comparison of serum inducible nitric oxide synthase (INOS) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

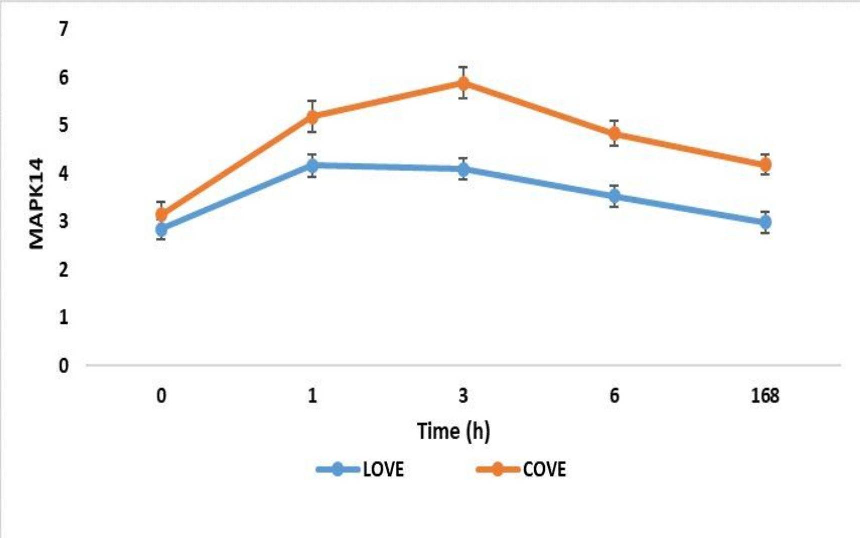

Figure 8 Comparison of serum mitogen activated protein kinase (MAPK14) concentrations after laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

Table 1 Pre (0 h) and postoperative (1 h, 3 h, 6 h, and 7 days) serum cortisol, TNF-α, IL-6, CRP, Hp, MAPK14, and INOS levels (Mean±SE) of laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

Parameter	Group	0 hour	1 hour	3 hour	6 hour	7 day
Cortisol	LOVE	118 ± 6.2	170 ± 8.1	209 ±14.6	145 ±10.1	121± 8.0
	COVE	119 ± 6.2	244 ± 16.8	251 ± 13.6	163 ±12.5	126 ± 5.9
TNF-α	LOVE	1.24 ± 0.21	3.56 ± 0.33	3.14 ± 0.33	1.79 ± 0.25	1.24 ± 0.17
	COVE	1.73 ± 0.24	4.83 ± 0.41	5.04 ± 0.38	2.70 ± 0.31	1.87 ± 0.21
IL-6	LOVE	1.77 ± 0.18	3.75 ± 0.36	3.61 ± 0.41	2.22 ± 0.22	1.74 ± 0.16
	COVE	2.53 ± 0.18	4.12 ± 0.36	4.96 ± 0.41	3.29 ± 0.22	2.66 ± 0.16
Hp	LOVE	47 ± 2.02	59.3 ± 2.52	60.3 ± 2.16	54.8 ± 2.20	50.3 ± 2.32
	COVE	48 ± 2.02	63.4 ± 2.52	68.1 ± 2.16	59.9 ± 2.20	54.4 ± 2.32
CRP	LOVE	1.62 ± 0.12	2.74 ± 0.20	2.67 ± 0.20	2.20 ± 0.15	1.86 ± 0.14
	COVE	2.08 ± 0.12	3.38 ± 0.20	3.95 ± 0.20	3.34 ± 0.15	2.58 ± 0.14
INOS	LOVE	2.95 ± 0.34	5.02 ± 0.49	5.95 ± 0.60	5.15 ± 0.45	3.71 ± 0.44
	COVE	3.88 ± 0.34	6.18 ± 0.49	7.16 ± 0.60	6.42 ± 0.45	4.37 ± 0.44
MAPK14	LOVE	2.85 ± 0.23	4.17 ± 0.29	4.10 ± 0.28	3.53 ± 0.23	2.99 ± 0.22
	COVE	3.51 ± 0.23	5.19 ± 0.29	5.89 ± 0.28	4.84 ± 0.23	4.19 ± 0.22

TNF-α, Tumor Necrosis Factor Alpha: IL-6, Interleukin 6: Hp, haptoglobin: CRP, C-reactive protein: INOS, inducible nitric oxide synthase: MAPK14, inducible nitric oxide synthase.

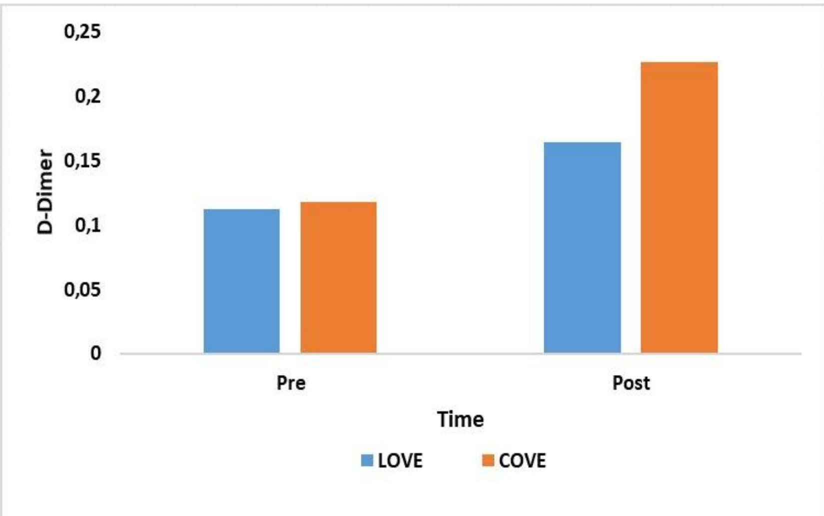

Figure 9 Preoperative and postoperative plasma levels of D-dimer laparoscopic (LOVE) and conventional open ovariectomy (COVE) in bitches.

Discussion

Laparoscopic or conventional open ovariectomy are the two main methods used for the sterilization of female dogs (Gower and Mayhew, 2008). With the development of the laparoscopic surgery technique, laparoscopic OV method has become one of the most common clinical methods of laparoscopy in the field of veterinary medicine (Dutta *et al.*, 2010).

A different scoring system to assess pain, such as the pain scale developed by the University of Melbourne, is used in veterinary medicine (Mich and Hellyer, 2008). It has been reported that UMPS is more advantageous compared with other pain tests, due to the presentation of quantitative data in terms of behavioral and physiological parameters and the fact that the observer's experience and interpretation are limited (Gültekin and Koç, 2012). Therefore, UMPS was used in that study to assess the postoperative pain score. In our study, we observed that the pain scores were lower in the LOVE compared to the COVE group, parallel to the other studies (Davidson *et al.*, 2004; Devitt *et al.*, 2005; Hancock, 2005).

There are reports concluding that the duration of the operation affect the pain and stress parameters in the postoperative period (Case *et al.*, 2011). In line with the literature (Shariati *et al.*, 2014), it was also observed in our study that the duration of the operation affects postoperative pain and the stress parameters. Contrary to our findings, there are studies suggesting that laparoscopic surgical procedures take longer than the conventional open surgical procedure (Hancock, 2005; Culp *et al.*, 2009; Del Romero *et al.*, 2020). Unlike our study, one or two-port laparoscopic techniques were used in dogs in these studies (Hancock, 2005; Culp *et al.*, 2009; Del Romero *et al.*, 2020). The operation site is more limited in the one or two-port LOVE methods performed by a single operator, and it is necessary to perform transabdominal ovarian fixation (Case *et al.*, 2011). It has been reported that transabdominal ovarian fixation is a difficult practice for the inexperienced operator (Nylund *et al.*, 2017). We consider that the reason why the operations in other studies (Hancock, 2005; Culp *et al.*, 2009; Del Romero *et al.*, 2020) take longer than the LOVE group in our study is that they were performed by a single operator and the need to perform transabdominal ovarian fixation. Furthermore, it has been reported that the duration of operation may vary in laparoscopic procedures, depending on the learning curve and experience (Davidson *et al.*, 2004; Devitt *et al.*, 2005; Mayhew and Brown, 2007; Culp *et al.*, 2009; Case *et al.*, 2011). Using three ports, having an assistant to help the operator and using two grasping forceps simultaneously (Pope and Knowles, 2014) instead of transabdominal ovarian fixation (Nylund *et al.*, 2017), which loses time, are considered the most significant factors for shortening the duration of the operation.

The duration of operation in the LOVE method may vary according to the sealing devices as well as the number of ports. In previous studies, it has reported that the LOVE method, in which vessel sealing is performed using LigaSure, shortens the duration of operation and ensures adequate hemostasis (Mayhew and Brown, 2007). Bipolar sealing is a more advantageous technique compared to monopolar sealing (Van Nimwegen *et al.*, 2005; Van Nimwegen and Kirpensteijn, 2007). In the current study, attempts were made to minimize the negative effects that could occur during the operation by using the electrothermal bipolar vessel sealing technique; an advantageous technique, in terms of operation duration and safety. We consider that the shorter duration of the three-port LOVE method, when compared with the laparoscopic ovariectomy techniques using one or two ports, is due to the reasons mentioned above.

Cortisol is frequently used in veterinary medicine for determining surgical stress (Devitt *et al.*, 2005; Hancock, 2005; Taves *et al.*, 2011). Cortisol levels, which are directly related with increasing pain, due to the severity of the surgical trauma, increase with the beginning of the operation (Marcovich *et al.*, 2001). Parallel to our findings, it has been reported in other studies (Marcovich *et al.*, 2001; Naitoh *et al.*, 2002) that the cortisol levels were lower in laparoscopic methods than in conventional open surgical methods. In order to quantitatively demonstrate the postoperative stress and pain, it is necessary to jointly assess the physiological and biochemical parameters (Hansen, 2003). Therefore, in our study the pain scores (UMPS) and cortisol levels of the dogs in both groups were jointly assessed. In our study, it was observed that the pain scores and cortisol levels in the LOVE group were lower compared to the COVE group, in accordance with the literature data (Devitt *et al.*, 2005; Hancock, 2005).

APR shows an increase as a result of the release of proinflammatory cytokines from damaged tissues due to the trauma (Cray, 2012; Sevgisunar and Şahinduran, 2014). Changes in the levels of proinflammatory cytokines (TNF-α and IL-6) and acute phase proteins (CRP, Hp) were compared to assess the surgical stress that increases due to surgical trauma.

For the purpose of this study, we selected IL-6 and TNF-α cytokines. The first is a primary mediator of the acute phase response in the peripheral blood and an early sensitive marker of tissue damage. Moreover, the elevation in serum IL-6 is proportional to surgical trauma and related injury (Cruickshank *et al.*, 1990). No study was found on the comparison of laparoscopic ovariectomy and conventional open ovariectomy methods in terms of proinflammatory cytokines. Therefore, we compared our findings with studies that compared different laparoscopic operations with conventional open surgical procedures. Parallel to our findings, it has been reported in other studies that IL-6 (Torres *et al.*, 2007; Martínek *et al.*, 2012) and TNF-α (Ordemann *et al.*, 2001; McGee *et al.*, 2008) levels were lower compared to conventional open surgical procedures.

Among acute phase proteins, CRP (Cray, 2012) and Hp (Rubio *et al.*, 2015) are parameters used for determining the severity of surgical trauma or inflammation (Christensen *et al.*, 2015). Consistent with the results of our study, it was reported that the CRP (Dąbrowski and Wawron, 2014; Dąbrowski *et al.*, 2015) and Hp (Alves *et al.*, 2010) levels were lower than conventional open surgical procedures. Hp levels increase, depending on the tissue damage (Aziz., 2012). In line with the study by Aziz (2012), it was observed that the Hp levels showed a higher increase compared to the initial levels in the COVE group than in the LOVE group, therefore there was higher tissue damage in the COVE group.

In another study (Del Romero *et al*., 2020), it was reported that the Hp level following the laparoscopic ovariectomy was higher compared to the conventional open surgical procedure, which was different from our findings. However, the researcher stated that the potential blood loss was not taken into consideration in the operations. As is known, Hp levels associated with free hemoglobin (Schmidt *et al*., 2018) can decrease due to hemorrhage or hemolysis (Del Romero *et al*., 2020). It is suggested that the loss of blood should be determined in order to assess the decreasing Hp levels (Del Romero *et al*., 2020). Therefore, we consider that the potential loss of hemoglobin during conventional open surgery can be a factor restricting the expected increase in Hp levels. The main purpose of DD analysis in our study was to assess the potential blood loss by determining the coagulation rates. DD concentrations were reported to increase in dogs due to surgical trauma (Schietroma *et al*., 2008). Schietroma *et al*. (2004) suggested that DD concentrations were higher in the postoperative period compared to the preoperative period, however the concentrations were higher in the conventional open surgical method at 1 hour post-operation compared to the laparoscopic method. The results of Schietroma *et al*. (2004) was in agreement with our findings. In the LOVE method, better viewing of the abdominal cavity and veins as well as less manipulation of the tissues reduce the risk of hemorrhage (Shariati *et al*., 2014). On the other hand, the COVE method can cause more bleeding due to a wider incision site and potentially faulty ligation. According to our results, higher DD concentrations in the COVE method indicates that the LOVE method is more reliable.

INOS is an enzyme which is synthesized under acute reactions such as trauma, stress and inflammation (Kuyumcu *et al*., 2004). INOS expression is one of the guidelines used for the assessment of APR which occurs in response to surgical stress, like IL-6 and CRP (Wichmann *et al*., 2005). These mediators are parameters which can be objectively and quantitatively used in the assessment of inflammatory system activation in response to surgical trauma (Chu *et al*., 2013). The laparoscopic method has been reported to have lower immune activation compared to the conventional open surgical method (Mutter and Aprahamian, 2000). No articles were found on the assessment of INOS in laparoscopic ovariectomy operations, therefore we compared our data with different laparoscopic surgical procedures. The results of Hajri *et al*. (2000) are in line with the results of our study. Compared to laparoscopic methods, it was reported that the expression of INOS was suppressed following conventional open surgical methods (Hajri *et al*., 2000). Chu *et al*. (2013) which compared transoral and transthoracic thoracoscopic surgery methods, it was reported that there were no differences between the groups in terms of INOS expression in the postoperative period. However, we consider that the LOVE method causes lower surgical stress and thus lower INOS levels, due to the fact that its potential to cause hemorrhages and its traumatic effects on ovarian ligaments are lower compared to the COVE method.

MAPK14 is a protein that is synthesized as a result of stress (Schäfer and Williams, 2000). There is a limited number of studies which have investigated the MAPK14 levels following surgical procedures in dogs. In a study that compared the conventional open surgery and laparoscopic procedure, it was reported that the MAPK14 levels were higher compared to the control group. However, no differences were found between the groups (Vittimberga *et al*., 2000). In our study, MAPK14 levels were lower in the LOVE method compared to the COVE method. Vittimberga *et al*. (2000) conducted an experimental study and did not carry out a procedure that could cause any surgical trauma. Therefore, we consider that the difference between the said study and our findings is caused by this.

One of the first cytokines that could be found in the blood following tissue damage or physical stress is TNF-α (Vilcek 2008). It was reported that cytokines like TNF-α stimulates INOS (Aktan, 2004) and MAPK14 (Lui *et al*., 2015). In our study, a positive correlation was observed between TNF-α and INOS and MAPK14. In our opinion, the fact that TNF-α, INOS and MAPK14 levels were lower in the LOVE group compared to the COVE group is caused by the lower occurrence of surgical trauma in the LOVE group. The lower postoperative UMPS scores in the LOVE group compared to the COVE group supports our hypothesis. Surgical stress and pain score measurements were repeated to assess the health statuses and postoperative activities of the dogs in both groups on day 7 following the operation. As expected, the surgical stress parameters and pain scores of all the animals were close to the preoperative levels on day 7 post-operation.

It was observed that the levels of APR, INOS, MAPK14, cortisol and DD, as well as the pain scores, were lower in the three-port LOVE method compared to the COVE method. Furthermore, we consider that the INOS and MAPK14 levels, which increase due to surgical stress, could contribute to other studies in this regard, due to our study being the first to compare the LOVE and COVE methods. This, in addition to previously demonstrated improved postoperative comfort and earlier return to normal function, means that laparoscopy could be considered preferable when performing ovariectomy in bitches. As a result, it was concluded that the LOVE (three port) was a minimally invasive method with shorter duration of operation, higher patient comfort with lower surgical stress and postoperative pain as well as positive results in terms of recovery period.

Conflict of Interest: No author declares a competing interest.

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author's doctoral dissertation prepared under the second author's supervision.

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