

**Surgical Time for Laparoscopic Ovariectomy in Adult and Prepubertal Dogs**Gizem TEZ<sup>1,a</sup>, Halit KANCA<sup>2,b,\*</sup>, Semra ERGUL<sup>1,c</sup><sup>1</sup>Ankara University, Institute of Health Sciences,  
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Available on-line at:

<https://dergipark.org.tr/tr/pub/huvfd>**Abstract:** Two-portal laparoscopic ovariectomy (LOVE) was performed in adult (n=23) and prepubertal dogs (n=23) to compare surgical time and to evaluate the effect of age, body weight (BW), body condition score (BCS), ovarian pedicle fat score (OPFS) and intraoperative surgical complications on surgical time. Three classes of BCS were considered: BCS 1-2=lean; BCS 3=ideal; BCS 4-5=overweight. The same surgeon performed all surgeries. OPF was scored and was considered negative or positive. Intraoperative complications were recorded. Total surgical time was recorded from the first skin incision to the last portal closure suture placement. The surgical procedure was divided into seven stages, and each stage's time was recorded. Data are reported as mean±SEM. Differences in the duration of defined surgical stages between groups were evaluated by age, BW, OPFS and intraoperative complications. One-way analysis of variance was used to assess the effect of BCS. No major complications were observed. Five and four minor complications were observed in adult and prepubertal dogs, respectively. Total surgical time was not different (p>0.05) between adult (20.63±0.60 min) and prepubertal (21.48±1.75 min) dogs. BCS, BW, and OPF did not affect total surgical time (p>0.05). Intraoperative complications have prolonged surgical procedure times (25.20±2.33 min vs 20.04±0.93 min). The time from the first skin incision to the insertion of the first trocar was shorter (p<0.05) in prepubertal dogs (2.38±0.12 min) compared to adult animals (2.71±0.10 min). The time needed for grasping of the right ovary was longer (p<0.05) in prepubertal dogs (3.02±0.31 vs 2.32±0.16 min) and in animals without OPF (2.94±0.24 vs 2.11±1.89 min). In conclusion, LOVE is safe in adult and prepubertal dogs with similar surgical time and complication rates.**Keywords:** Dog, Laparoscopy, Ovariectomy, Prepubertal, Surgical time.**Yetişkin ve Prepubertal Köpeklerde Laparoskopik Ovariectomi Cerrahi Süresi****Özet:** Cerrahi sürenin karşılaştırılması ve yaş, vücut ağırlığı (VA), vücut kondisyon skoru (VKS), ovarian pedikül yağ skoru (OPYS) ve intraoperatif cerrahi komplikasyonların cerrahi süre üzerine etkilerinin araştırılması amacıyla yetişkin (n=23) ve prepubertal köpeklerde (n=23) iki-portal laparoskopik ovariectomi (LOVE) yapılmıştır. Üç VKS sınıfı dikkate alınmıştır: VKS 1-2=zayıf; VKS 3=ideal; VKS 4-5=fazla kilolu. Tüm ameliyatlar aynı cerrah tarafından yapılmıştır. OPY skorlanmış ve negatif veya pozitif olarak kabul edilmiştir. İntraoperatif komplikasyonlar kaydedilmiştir. Toplam cerrahi süre, ilk deri ensizyonundan son portal kapatma sütürünün yerleştirilmesine kadar geçen süre olarak kaydedilmiştir. Cerrahi işlem 7 farklı aşamaya ayrılmış ve her aşamanın süresi kaydedilmiştir. Veriler ortalama±SEM olarak sunulmuştur. Gruplar arasında tanımlanmış cerrahi sürelerle yaş, VA, OPYS ve intraoperatif komplikasyonların etkisi ile değerlendirilmiştir. VKS etkisinin değerlendirilmesi için tek yönlü varyans analizi kullanılmıştır. Majör komplikasyon ile karşılaşılmamıştır. Erişkin ve prepubertal köpeklerde sırasıyla 5 ve 4 minör komplikasyon gözlenmiştir. Erişkin (20,63±0,60 dk) ve prepubertal (21,48±1,75 dk) köpekler arasında toplam cerrahi süre farklılık göstermemiştir (p>0,05). VKS, VA ve OPY toplam cerrahi süresini etkilememiştir (p>0,05). İntraoperatif komplikasyonlar cerrahi süreyi uzatmıştır (25,20±2,33 dk; 20,04±0,93 dk). İlk deri ensizyonundan ilk trokarın yerleştirilmesine kadar geçen süre prepubertal köpeklerde (2,38±0,12 dk) erişkin hayvanlara (2,71±0,10 dk) kıyasla daha kısa olmuştur (p<0,05). Sağ ovaryumu kavramak için gereken süre prepubertal köpeklerde (3,02±0,31 dk; 2,32±0,16 dk) ve OPY negatif hayvanlarda (2,94±0,24; 2,11±1,89 dk) daha uzun olmuştur (p<0,05). Sonuç olarak LOVE, benzer cerrahi süre ve komplikasyon oranları ile yetişkin ve prepubertal köpeklerde güvenli bulunmuştur.**Anahtar Kelimeler:** Cerrahi süre, Köpek, Laparoskopji, Ovariectomi, Prepubertal.

## Introduction

Elective sterilization in dogs is the most commonly performed surgical procedure in veterinary practice (DeTora and McCarthy, 2011; Tez et al., 2019). Many techniques for surgical sterilization in dogs have been described, each offering advantages and disadvantages to both the patient and surgeon. The surgeon has to choose the least invasive, fastest, and safest procedure. Technically, ovariectomy (OVE) can be performed with a smaller incision and less tissue trauma due to the absence of corpus uteri and suspended ligaments' disturbance. Thus, the surgical time for ovariectomy is shorter than for ovariohysterectomy (Tallant et al., 2016; Van Goethem et al., 2006).

Minimally invasive surgery has long been suggested as an alternative to traditional ovariohysterectomy or ovariectomy (Manassero and Viateau, 2018). With the development of minimally invasive surgical techniques, laparoscopic ovariectomy has gained popularity in veterinary medicine (Culp et al., 2009). In particular, increasing public awareness has led pet owners to adopt a less invasive approach to surgical interventions for their pets. In addition, the number of veterinarians who offer laparoscopic methods to their patients is increasing rapidly (Buote, 2022; Hsueh et al., 2018). Today, laparoscopic surgery in dogs is mainly applied for elective sterilization (Findji, 2014; Tez and Kanca, 2018).

The advantages of laparoscopic ovariectomy (LOVE) are better visibility, smaller incisions, reduced ovarian remnant syndrome risk, decreased surgical trauma, shortened healing time, atraumatic cauterization, and less surgical pain and stress. (Marvel, 2022). These advantages are promising when surgical time, hospitalization duration, complication rate, and cost-intensive sterilization programs are important. However, the cost of tools and equipment, potential intraoperative complications, and the need for specific training limit their widespread use (Tez and Kanca, 2018).

The term prepubertal is generally used for dogs from 6 to 14 weeks, although it defines the period before the onset of sexual activity (Stubbs and Bloomberg, 1995). Prepubertal sterilization is routinely performed in animal rehabilitation centers, especially in the United States and the United Kingdom, because it is considered the easiest and most effective population control method (Moxon et al., 2023). Many veterinarians and institutions advocate prepubertal or pre-adoption spaying to prevent the rapid population increase, maintenance and hosting cost reduction, and the risk of mammary neoplasia in dogs (Howe et al., 2001; Valdez, 2022). Even so, there is an ongoing dilemma about shortening surgical time through better visibility or small size of reproductive tissues and high tissue fragility causing harsh procedures (Howe, 2006; Stubbs and Bloomberg, 1995).

Laparoscopic OVE has not been extensively studied in prepubertal dogs, and controlled studies comparing the duration of surgical sterilization of prepubertal and adult female dogs are warranted. Therefore, we aimed to investigate the effects of body weight, body condition score, ovarian pedicle fat status, and intraoperative complications

on laparoscopic ovariectomy surgical time in both prepubertal and adult dogs.

## Material and Methods

The study procedure was approved by the Ankara University Local Ethics Committee of Animal Experiments (No: 2016-5-53). We used 46 (23 adult and 23 prepubertal) female dogs weighing 8-44 kg. Anamnesis, physical examination, complete blood count, abdominal ultrasonography, and vaginal cytology were used to determine the suitability of all dogs for surgical sterilization. Vaginal smear samples were taken from adult dogs to determine the sexual cycle stage. The sexual cycle stage was determined in smear samples stained with the Papanicolaou method (Feldman and Nelson, 2004). Adult dogs out of the follicular phase and prepubertal were included.

All surgical procedures were performed under the same anesthesia and analgesia protocol. A single dose of cefazolin (20 mg/kg, IM) was applied 30 min before surgery. All dogs were premedicated with medetomidine hydrochloride (0.04 mg/kg). For the induction of anesthesia, propofol (2-3 mg/kg) was administered intravenously (IV). After intubation, general anesthesia was maintained with isoflurane (1%, Isoflurane) in 50% oxygen.

The dogs were placed in the dorsal recumbent position on a special operating table that could tilt to the right and left. The ventral abdomen was shaved from the xiphoidal region to the pubis, and the urinary bladder was examined by ultrasonography. In cases where the bladder is full enough to affect the operation's success, the urine is emptied by abdominal compression or catheterization. After cleaning the operation area with soapy water, asepsis was provided with povidone-iodine.

One surgeon (G.T.) performed all surgeries. All dogs received lactated ringer solution (5 ml/kg/hr, IV) and were monitored during surgery. A two-portal technique and an electrothermal bipolar vessel sealing device (LigaSure™) were used. A 7 mm skin incision was made 2 cm caudal to the umbilicus. The first 5 mm trocar (VersaOne™ Bladed Trocar, Covidien, Turkey) was inserted using Hasson's technique. Pneumoperitoneum was achieved with pressure of 10-12 mmHg. The second trocar (11-12 mm, VersaOne™ Bladed Trocar, Covidien, Turkey) was inserted through a skin incision halfway between the first incision and the pubis. A 5 mm diameter, 30° angle of a laparoscope (30 cm, Eickemeyer®, Germany) was introduced into the cranial cannula, and a grasping forceps (5 mm, 36 cm, Eickemeyer®, Germany) into the caudal cannula. The left ovary was grasped and pulled to the abdominal wall with the dog in the right lateral recumbency. A transabdominal suspension suture was used to fix the ovary. The associated ligaments were sealed and transected using a laparoscopic vessel sealing device (LigaSure™, 5 mm, 37 cm, Blunt Tip, Covidien, Turkey). The ovary was removed from the abdomen through the portal. The dog was positioned in left lateral recumbency, and the procedure was repeated for the right

ovary. Pneumoperitoneum was released, and abdominal portals were closed in 2 layers.

Body weight (BW), body condition score (BCS), ovarian pedicle fat score (OPFS), and intraoperative complications were recorded. Body condition was scored using a 1-5 point BCS system (Impellizzeri et al., 2000). The dogs were grouped as lean (BCS 1-2), ideal (BCS 3), and overweight (BCS 4-5). Ovarian pedicle fat was scored during operations (Van Nimwegen and Kirpensteijn, 2007) from 0 to 3, and OPF status was considered negative (OPFS= 0-1) or positive (OPFS=2-3).

Intraoperative complications were recorded as minor or major. Bleeding from the spleen, ovarian bursa, pedicle or other iatrogenic abdominal trauma were characterized as minor intraoperative complications. Major complications were evaluated as requiring intraoperative conversion to laparotomy or postoperative revision.

In order to compare the surgical times between the groups, the total time and the times for all surgical stages were recorded separately. Total surgical time was recorded from the first skin incision to the last portal closure. The surgical procedure was divided into seven stages, and the time for each step was recorded: Skin incision until first trocar insertion (1); insertion of second trocar (2); grasping of left ovary (3); removal of left ovary (4); grasping of right ovary (5); removal of right ovary (6); abdominal portal closure (7).

The Student's t-test was used to examine the effects of age (prepubertal and adult) and OPF on surgical time and to

compare surgical times. One-way analysis of variance (ANOVA) was used to examine the differences in surgical time in terms of study groups and BCS groups. Tukey's test was applied as a post hoc test for groups that were found to be significant. Descriptive statistics on surgical time and demographic findings are shown as "Arithmetic Mean±Standard Error". Pearson correlation analysis was used to determine the strength and direction of the relationship between surgery time and body weight. SPSS 14.01 (License No: 9869264) package program was used to analyze the data. The  $p<0.05$  criterion was taken into account in all statistical decisions.

## Results

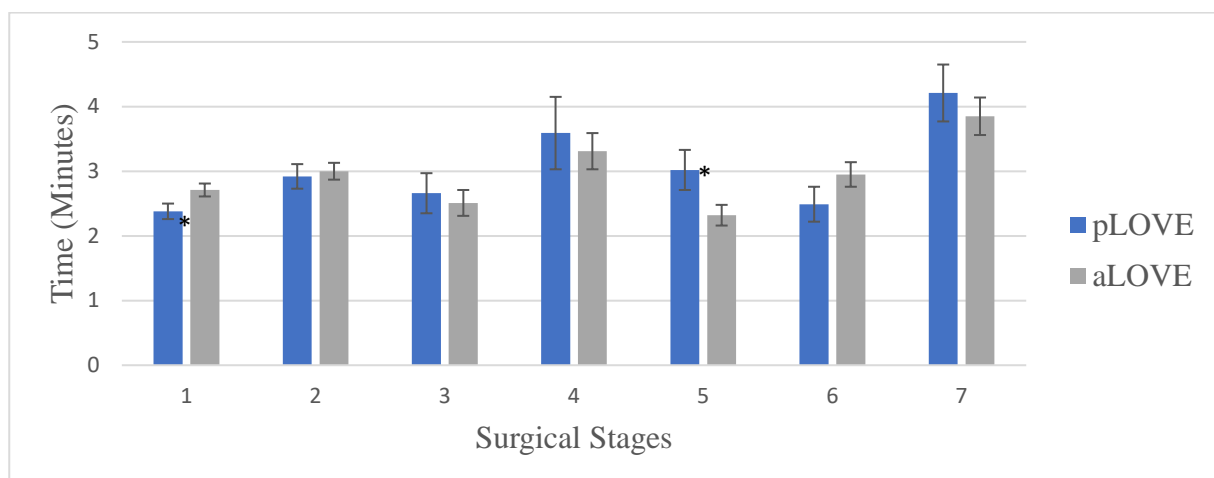
The mean age of the animals used in the study was  $6.35\pm0.04$  months (5-8 months) in prepubertal dogs and  $21.17\pm0.36$  months (10-48 months) in adult dogs. In prepubertal and adult dogs the mean body weight was  $15.39\pm0.18$  kg (9-27 kg) and  $23.35\pm0.36$  kg (13-42 kg). Most prepubertal dogs ( $n=10$ ) had a BCS of 3. Ten prepubertal dogs were classified as lean. A BCS of 5 was not observed in prepubertal dogs. However, three dogs were classified as overweight. Three adult dogs were lean. The body condition of 14 adult dogs was ideal, and six dogs were overweight. Only two prepubertal dogs have ovarian pedicle fat. In contrast, 13 adult dogs were classified as OPF-positive. The mean age, BW, BCS, and OPFS of dogs in study groups are presented in Table 1.

**Table 1.** The mean age, body weight, body condition score and ovarian pedicle fat score in prepubertal and adult dogs.

Group	n	Age (month)	Body Weight (kg)	Body Condition Score (1-5)	Ovarian Pedicle Fat Score (0-3)
pLOVE <sup>1</sup>	23	$6.35\pm0.04$	$15.39\pm0.18$	$2.52\pm0.04$	$0.44\pm0.03$
aLOVE <sup>2</sup>	23	$21.17\pm0.36$	$23.35\pm0.36$	$3.17\pm0.36$	$1.74\pm0.04$

<sup>1</sup>Prepubertal laparoscopic ovariectomy.

<sup>2</sup>Adult laparoscopic ovariectomy.



**Figure 1.** Surgical stage times during laparoscopic ovariectomy in prepubertal and adult dogs.

1<sup>st</sup> stage: skin incision until first trocar insertion; 2<sup>nd</sup> stage: insertion of second trocar; 3<sup>rd</sup> stage: grasping of left ovary; 4<sup>th</sup> stage: removal of left ovary; 5<sup>th</sup> stage: grasping of right ovary; 6<sup>th</sup> stage: removal of right ovary; 7<sup>th</sup> stage: abdominal portal closure. \* $p<0.05$ : represents statistically significant difference among groups in the same surgical stage. pLOVE: prepubertal laparoscopic ovariectomy; aLOVE: adult laparoscopic ovariectomy.

No difference was observed in total surgical time between prepubertal (pLOVE: 21.48±1.75 min) and adult (aLOVE: 20.63±0.60 min) dogs ( $p>0.05$ ). Closure of the portals was the most time-consuming stage during LOVE (pLOVE: 4.21±0.44 min; aLOVE: 3.85±0.29 min,  $p>0.05$ ). In pLOVE, the time needed for the first trocar insertion was shorter ( $p=0.044$ ); in contrast, the time required for grasping the right ovary was longer than aLOVE ( $p<0.05$ ). The time needed for insertion of the second trocar (pLOVE: 2.92±0.19 min; aLOVE: 3.00±0.13 min), grasping of left ovary (pLOVE: 2.66±0.31 min; aLOVE: 2.51±0.20 min), removal of left ovary (pLOVE: 3.59±0.56 min; aLOVE: 3.31±0.28 min) and removal of right ovary (pLOVE: 2.49±0.27 min; aLOVE: 2.95±0.19 min) did not differ among the groups ( $p>0.05$ ). A comparison of surgical stage times between prepubertal and adult LOVE is given in Figure 1.

No relationship was observed between body weight and total surgical time ( $p=0.37$ ). Laparoscopic OVE total surgical time and surgical stage times were not affected by BCS ( $p>0.05$ ). Total surgical time in lean, ideal, and overweight dogs were 18.45±1.27 min, 22.82±1.52 min, and 20.10±0.83 min, respectively. Total surgical times of LOVE were not affected by OPF ( $p>0.05$ ). Total surgical time was 20.04±0.57 min in OPF (+) dogs and 21.54±1.33 min in OPF (-) dogs. However, the grasping of the right ovary during LOVE was shorter ( $p=0.029$ ) in OPF-positive dogs (2.11±1.89 min) compared to OPF-negative dogs (2.94±0.24).

A total of 9 minor intraoperative complications were identified (19.6%), and no major intraoperative complications were observed during LOVE. All intraoperative complications were minor haemorrhagia due to tissue damage originating from intestines ( $n=1$ ; 2.2%), urinary vesicle ( $n=1$ ; 2.2%), spleen ( $n=3$ ; 6.6%) and ovarian pedicle ( $n=4$ ; 8.7%) haemorrhagia. Only for ovarian pedicle haemorrhagia Ligasure™ (Covidien, Medtronic, Türkiye) was used: none others needed intervention.

Total surgical time was longer in the presence of intraoperative complications (20.04±0.93 min vs. 25.20±2.33 min;  $p=0.024$ ). Time for the second trocar insertion was longer in LOVE with intraoperative complications (3.54±0.22 min) when compared to LOVE (2.82±0.12 min) without complications ( $p=0.009$ ). Intraoperative complications did not affect other surgical stage times of LOVE ( $p>0.05$ ).

## Discussion and Conclusion

Laparoscopic OVE surgery time is affected by several factors, such as the number of ports, pneumoperitoneum induction method, imaging system, hemostasis or resection method, and operator experience. Consequently, different studies reported varying surgical times for LOVE (Freeman et al., 2010; Van Nimwegen and Kirpensteijn, 2007). In a study investigating the effects of the number of ports, total surgical times for single and double portal LOVE were similar. However, the duration of surgery extended in three portal techniques (Case et al., 2011). The use of a single port in laparoscopic OVE provides an aesthetic advantage (Manassero et al., 2012); however, it is disadvantageous compared to the double portal technique in terms of

maneuverability and ease of access (Case et al., 2011; Dupre et al., 2009). Therefore, the most commonly used method in dogs is two-portal LOVE.

As an alternative to mechanical methods (ligature, clip, staple) for hemostasis during LOVE in dogs, monopolar and bipolar electrocoagulation (Van Goethem et al., 2003), laser technology (Van Nimwegen and Kirpensteijn, 2007; Van Nimwegen et al., 2005), ultrasonic devices (Hancock et al., 2005), and electrothermal bipolar vessel sealing instruments (Culp et al., 2009; Dupre et al., 2009; Mayhew and Brown, 2007) have been reported. The current hemostasis method recommended for LOVE in dogs is electrothermal bipolar vessel sealing (Culp et al., 2009; Öhlund et al., 2011). In the present study, a two-portal technique and an electrothermal bipolar vessel sealing device (Ligasure™, Covidien, Medtronic, Turkey) were chosen to minimize the possible negative effects on surgical times. As a result, LOVE surgical times were shorter than previously reported studies using different port numbers and instrumentation (Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007; Van Nimwegen et al., 2005). The LigaSure™ system sends a warning signal when coagulation is complete, and it shortens the surgical time by allowing the operator to cut manually and remove the ovaries without changing instruments in between. In addition, Ligasure™ causes less damage to the surrounding tissues by creating a heat dissipation of up to 1.5 mm, reducing the rate of intraoperative complications (Culp et al., 2009; Öhlund et al., 2011). Similar LOVE total surgery times have been reported in studies using the same technique. Case et al. (2011) reported that LOVE was completed in 19.1±6.3 min. In a Dupre et al. (2009) study, the total surgical time was 18.2±4.4 min.

It has been suggested that the surgical time will be shortened due to better visibility in prepubertal sterilization (Stubbs and Bloomberg, 1995). On the other hand, small reproductive tissue sizes and high tissue fragility can adversely affect the total surgical time. Although total surgical times were not different in the current study, inserting of the first trocar was shorter in prepubertal dogs. Conversely, the time needed for grasping the right ovary was longer. The incision time of the thin abdominal layers is less in prepubertal dogs. The current study used the open technique to insert the first trocar. In this technique, abdominal muscle and skin incisions can be performed more quickly in prepubertal dogs. In addition, the presence of subcutaneous adipose tissue in adult dogs may cause prolongation of the surgical stage.

It has been reported that it is more difficult to reach the ovaries in dogs under one year of age during LOVE, and the sexual cycle stage plays an essential role in detecting the ovaries (Van Nimwegen and Kirpensteijn, 2007). The faster detection of the right ovary, located more cranially in adult dogs, is attributed to the larger uterine and ovarian tissue and reproductive hormone exposure. In the current study, vaginal cytological examination was performed, and dogs in the follicular phase of the sexual cycle were omitted. Therefore, the authors believe there was no effect of the sexual cycle stage on surgical time. The authors further believe that controlled ligation of the thin and sensitive

ovarian pedicle in prepubertal dogs requires more time to remove the more cranially located right ovary.

Body weight did not affect the total surgical time in any of the study groups. In two studies evaluating the effects of body weight on the total surgical time of LOVE, no significant effect was observed (Öhlund et al., 2011; Tapia-Araya et al., 2015). On the other hand, Dupre et al. (2009) reported that body weight affects the total surgical time, and surgery is prolonged in dogs with a body weight of <7 kg and >33 kilograms. Body weight is a criterion for identifying dogs suitable for LOVE. Dogs weighing >8-10 kg were used in almost all studies (Hancock et al., 2005; Mayhew and Brown, 2007; Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007). In addition, a prerequisite of >8 kg is required for dogs to be treated with LOVE at the Royal Veterinary College. In dogs with a body weight of >33 kilograms, it was reported that the duration of surgery was prolonged due to the inadequacy of hand instruments with a working length of 37 cm, and the use of longer laparoscopic instruments has been suggested (Dupre et al., 2009). In the current study, 37 cm long hand instruments were used successfully.

In the current study, BCS did not affect total surgical time and surgical stage times. Two studies using techniques similar to the present study reported that BCS did not affect the total surgical time of LOVE (Öhlund et al., 2011; Tapia-Araya et al., 2015). It has been suggested that LOVE may be an alternative to traditional OVE by visualizing the ovaries in obese dogs with high abdominal fat and requiring wide incisions (Van Goethem et al., 2003). Unaffected LOVE times in the current study reveal the advantage of LOVE in the surgical sterilization of obese dogs and support the abovementioned suggestion.

Ovarian pedicle fat is considered the most important factor affecting the duration of surgery (Harris et al., 2013). The presence of OPF increases the total surgical time of canine LOVE using laser and bipolar electrocoagulation methods (Van Nimwegen and Kirpensteijn, 2007), and the total duration of surgery and the duration of the surgical stage was longer in small-sized dogs with high OPFS (Granados et al., 2017). However, the total surgical time was not affected by OPY in two studies with Ligasure™ (Öhlund et al., 2011; Tapia-Araya et al., 2015). In the current study, LOVE total surgery time was unaffected by OPF, and the time needed to identify the right ovary was shorter in dogs with OPF. The lacking effect of OPF on the total surgical time of LOVE has been associated with the ease in hemostasis of ovaries in the current study. The shorter time needed to identify the right ovary in dogs with OPF may be attributed to periovarian fat resulting in better visibility.

No major complications were observed during LOVE in the current study. This result is consistent with the fact that no major complications requiring conversion to open surgery were reported in the last 20 years (Case et al., 2011; Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007). No major intraoperative complications were reported in a large retrospective study of 618 LOVE, (Pope and Knowles, 2014). More strikingly, no major complications were observed in 161 LOVE performed by Colorado State

University fourth-year students (Nylund et al., 2017). As a result, LOVE is a very safe method for intraoperative complications for both prepubertal and adult dogs. In addition, the availability of open surgery when necessary is an essential advantage for LOVE.

Intraoperative complications in the study resulted in longer total surgery times. In terms of surgical stage times, only the time taken for insertion of the second trocar was affected by intraoperative complications. Complications during laparoscopic sterilization are directly related to the suitability of the equipment used and the operator's experience. Intraoperative complications during LOVE using Ligasure™ usually occur due to minor hemorrhage, do not require intervention, and hemostasis is achieved spontaneously (Culp et al., 2009). Furthermore, it is possible to intervene in hemorrhages.

It has been concluded that LOVE is a safe method for surgical sterilization of prepubertal and adult dogs. Total surgery time is similar in prepubertal and adult dogs and is not affected by body weight and body condition or OPF. Furthermore, grasping of the right ovary during LOVE is shorter in dogs with OPF. Minor intraoperative complications prolong the total surgical times of LOVE by increasing the time needed for the second trocar insertion.

### Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

### Ethical Approval

This study was approved by the Ankara University Animal Experiments Local Ethics Committee (24.02.2016, 2016-5-53 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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### Similarity Rate

We declare that the similarity rate of the article is 15% as stated in the report uploaded to the system.

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### Explanation

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## Author Contributions

Motivation / Concept: GT, HK  
 Design: GT, HK  
 Control/Supervision: GT, HK  
 Data Collection and / or Processing: GT, HK  
 Analysis and / or Interpretation: GT, HK  
 Literature Review: GT, HK  
 Writing the Article: GT, HK, SE  
 Critical Review: SE

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