

Effects of Surgical Skills of Veterinary Medicine Students on Postoperative Stress, Oxidative Stress and Pain in Bitches Undergoing Ovariohysterectomy

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ABSTRACT

This study was designed to demonstrate the effects of surgical skills of final-year veterinary medicine students relating to total surgery time and skin incision length on postoperative stress, oxidative stress and pain in bitches undergoing ovariohysterectomy under identical anaesthesia and surgical procedures. The control group (n=12) consisted of bitches that were operated on by an experienced surgeon, while the ovariohysterectomies in the experiment group (n=12) were performed by final-year veterinary medicine students. Blood samples were taken 24 hours before the surgery, during premedication, at the end of surgery and 30 minutes, 1, 2, 4 and 6 hours after surgery for the analysis of cortisol, total oxidant status (TOS), total antioxidant status (TAS) and for the calculation of the oxidative stress index (OSI). Modified Melbourne Pain Scale was used for pain assessment. It was observed that the total surgery time and length of skin incision in the experiment group were significantly higher ($p<0.001$) than those detected in the control group. The concentrations of cortisol were statistically higher ($p<0.001$) in the experiment group than those measured in the control group, apart from the concentrations measured at 24 hours before surgery. The concentrations of TOS and TAS did not show any significant differences within and between groups. Similarly, OSI did not differ within groups. However, OSI values at premedication time in the experiment group were statistically higher ($p<0.05$) than those calculated in the control group. Pain scores did not differ within and between groups. In conclusion, it is indicated that the blood cortisol concentrations tend to increase due to the surgery time or the length of skin incision. Moreover, ovariohysterectomies may be safely performed by final-year veterinary medicine students without any postoperative pain.

Keywords: Cortisol, dog, pain, TOS, TAS.

Veteriner Hekimliği Öğrencilerinin Cerrahi Becerilerinin Ovaryohistektomi Yapılan Köpeklerde Postoperatif Stres, Oksidatif Stres ve Ağrı Üzerine Etkileri

ÖZ

Sunulan çalışmada, veteriner hekimliği eğitiminin son sınıfında olan öğrencilerin, toplam operasyon süresi ve deri ensizyonu uzunluğu ile ilişkili olan cerrahi becerilerinin operasyon sonrası stres, oksidatif stres ve ağrı üzerine etkilerinin aynı anestezi ve cerrahi prosedürler altında ortaya konulması amaçlandı. Tecrübeli bir hekimin opere ettiği köpekler kontrol grubunu (n=12) oluştururken, veteriner fakültesi son sınıfta eğitim gören öğrenciler tarafından opere edilen köpekler (n=12) deneme grubunu oluşturdu. Operasyondan 24 saat önce, premedikasyon sırasında, operasyon sonunda ve operasyondan 30 dakika, 1, 2, 4 ve 6 saat sonra kan örnekleri toplanarak kortizol, toplam oksidant durum (TOD), toplam antioksidant durum (TAD) ölçüldü ve oksidatif stress indeksi (OSI) hesaplandı. Modifiye edilmiş Melbourne Ağrı Skalası ağrı değerlendirilmesi için kullanıldı. Deneme grubundaki köpeklerde, toplam operasyon süresi ve deri ensizyon uzunluğunun kontrol grubundaki köpeklerden istatistiksel olarak ($p<0.001$) daha yüksek olduğu gözlemlendi. Deneme grubunda ölçülen kortizol düzeylerinin kontrol grubuna göre istatistiksel olarak yüksek ($p<0.001$) olduğu, ancak operasyondan 24 saat önceki değerlerde farklılık olmadığı belirlendi. TOD ve TAD düzeylerinin gruplar içinde ve arasında önemli farklılık oluşturmadığı izlendi. Benzer şekilde, OSI değerleri de gruplar içinde farklılık oluşturmadı. Bununla birlikte, deneme grubunda premedikasyon sırasındaki OSI değerlerinin kontrol grubuna göre istatistiksel olarak yüksek ($p<0.05$) olduğu gözlemlendi. Ağrı skorları ise gruplar içinde ve arasında farklılık oluşturmadı. Sonuç olarak, operasyon süresi ve deri ensizyonu uzunluğu ile ilişkili olarak kortizol düzeylerinin artış eğiliminde olduğu belirlendi. Diğer taraftan, veteriner hekimliği son sınıf öğrencilerinin de operasyon sonrası ağrıya neden olmadan güvenli bir şekilde kısırlaştırma operasyonları gerçekleştirebileceği düşünülmektedir.

Anahtar kelimeler: Kortizol, köpek, ağrı, TOD, TAD.

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INTRODUCTION

Upon their first employment, newly graduated veterinary surgeons are required to be competent in performing ovariohysterectomy procedures (OVH) (Hill et al., 2012), since OVH is one of the most common surgical practices in veterinary medicine. The European Coordination Committee for Veterinary Training (ECCVT) declares the directive 'Day One Competence' which describes the minimum standard required and is the starting point for a variety of roles in the veterinary profession (ECCVT, 2015). The surgical procedure of ovariohysterectomy does not only induce the oxidative stress and inflammatory process which might lead to short or long term side effects (Kucukakin et al., 200) but also causes the postoperative pain (Devitt et al 2005). The main problems facing newly-graduated veterinary surgeons are a lack of confidence and competence in basic skills, thus leading to the possibility of stressful initiations to their professional careers (Bowl et al., 2011). The OVH is an excellent teaching procedure for veterinary students (Zeugschmidt et al., 2016) due to its surgical complexity that requires basic knowledge about abdominal cavity anatomy, anaesthesia, antisepsis procedures, surgical instruments, suturing and surgical handling (Fossum., 2007). However, many veterinary medicine students (Langebaek et al., 2012) find the OVH surgical procedure to be challenging and stressful due to lack of self-confidence and experience. OVH is also very commonly the first live surgery experience for students of veterinary medicine and research has indicated that many students undertaking training in OVH report negative emotions such as nervousness, apprehension and stress (Langebaek et al., 2012). Surgical stressors and their impacts can influence the judgment, decision making and communication skills of junior surgeons who may show uncertainty about their ability to cope (Wetzel et al., 2006) leading to increased surgery durations and complication rates (Blacklock et al., 2016). Positive correlations between longer durations of surgery and anaesthesia and the incidence of wound infection, as well as teaching costs, have been demonstrated (Brown et al., 1997). Indeed, the biggest two concerns for final year students performing desexing surgeries are to finish the surgery in a reasonable amount of time (Gates et al., 2018) and to manage any postoperative bleeding (Blacklock et al., 2016; Gates et al., 2018). In contrast, the management of postoperative pain is of much less concern in performing these surgeries (Gates et al., 2018). In addition, evaluations of the attitudes of graduating veterinary students show that the clear definition of the recognition and the treatment of pain in animals is not sufficient (Hellyer et al., 1999). It has been reported that surgery time is inversely related to veterinary student experience, in that the time decreases, as their level of experience increases (Freeman et al., 2017). Since OVH has been an excellent teaching model for surgical procedures as

mentioned, this raises the question whether or not surgical manipulations in OVH procedures performed by veterinary medicine students may cause undue postoperative stress, oxidative stress and pain. Therefore, this study was designed to demonstrate how the surgical skills of final year veterinary medical students relating to total surgery time and skin incision length correspond to postoperative stress, oxidative stress and pain in bitches undergoing OVH.

MATERIALS and METHODS

A total of 24 bitches of different breeds which had been referred to the Afyon Kocatepe University animal hospital for elective OVH, were used in the study. The animals were aged 1 – 3 years and weighed 25 ± 2.1 kg on average. All procedures were approved by the local ethics committee of Afyon Kocatepe University (AKUHADYEK-156-16). The bitches were hospitalised for surveillance one day before the elective OVH at the animal hospital and were fasted for 8 hours with water consumption. First blood sampling and handling 24 hours prior to surgery were performed by the experienced veterinary surgeon.

The dogs were randomly divided into two equal groups. All surgical procedures, except anaesthesia and blood sampling but including fixing the patient to the surgery table, transferring the patient to the hospitalisation unit and OVH surgery in the experiment group ($n = 12$) were performed by veterinary students who were class participants in the last term of final-year. One student took the lead role as surgeon, whereas the other student was the assisting surgeon. All students had received training in procedures including handling protocols, surgical preparations and surgical protocols for OVH in their fourth and fifth-year classes. All students had performed both assisting and leading roles in OVH surgery on bitches before this study. Surgeries in the control group ($n = 12$) were performed by one experienced surgeon (MA) with one assisting student. This same experienced surgeon performed all anaesthesia and blood sampling in both groups. Moreover, all surgeries were instructed by a lecturer surgeon (OY).

Anaesthesia and Surgery

A standard anaesthesia protocol was used for the dogs in both the control and experiment groups as previously described elsewhere (Korkmaz et al., 2019). Accordingly, atropine sulphate (subcutaneous, 0.045 mg/kg, Atropin, Deva Holding, Istanbul, Turkey) was administered approximately 30 minutes before general anaesthesia. Midazolam (intravenous, 0.3 mg/kg, Dormicum, Roche, Istanbul, Turkey) was injected for preanaesthetic medication. Meloxicam (subcutaneous, 0.2 mg/kg, Maxicam, Sanovel, Turkey) was injected following premedication. After sedation, anaesthesia was induced with propofol (intravenous, Propofol, 4 mg/kg, Fresenius Kabi, Istanbul, Turkey). The dogs

were orotracheally intubated using cuffed endotracheal tubes and connected to the automatic anaesthesia machine (SMS 2000, SMS Medical, Ankara, Turkey). General anaesthesia was maintained by administration of 2 % isoflurane (Forane, Abbott Laboratories, Istanbul, Turkey). Intravenous lactated Ringer's solution (10 ml/kg/h) was provided throughout the procedure. All dogs were monitored (Petas KMA 800, Profesyonel Elektronik Sanayi ve Ticaret, Ankara, Turkey) for their heartbeat and respiratory rates.

The ventral abdomen was prepared aseptically for surgery following the general anaesthesia. Briefly, a ventral midline skin incision was performed at the middle third of the umbilicopubic distance. After reaching the abdominal cavity, the uterine ligament was attached to a uterine hook to hold the uterus. Each ligamentum suspensorium ovarii was dissected by hand. The ovarian pedicles were ligated and then the ovaries were removed. The uterine body was separated from the broad ligament. Additional ligation was performed on the broad ligament, if bleeding was evident. After the ligation of the proximal part of the cervix uteri, the abdominal wall and the skin were closed. All data were recorded by the same researcher during each surgical procedure. The time between the start of skin incision and placement of the last skin suture was defined as total surgery time. The length of the ventral midline skin incision was measured by a caliper at the end of each surgery.

The students were observed by the same person who recorded the starting and finishing times of surgery. The data recorded was not shared with students so as to reduce the potential impacts of competitive stress on their performance. Moreover, students were set free for decision making and advice was not provided unless they specifically requested an opinion from the observer. The length of skin incision data were also not shared with students during the surgeries. Each dog received daily injections of penicillin + streptomycin (intramuscular, 20 mg/kg, IM Penoksal, Vilsan, Istanbul, Turkey) for five days. The sutures were removed 10 days after surgery.

Blood Sampling

Blood samples were collected to measure serum cortisol concentrations, total oxidant status (TOS) and total antioxidant status (TAS) 24 hours prior to surgery (T-24), during premedication (T), at the end of surgery (T0), 30 minutes after surgery (T^{1/2}) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes and then sera were stored at -20°C until further analysis of cortisol (ng/mL), TOS (μmol H₂O₂ Eq/L) and TAS (mmol Trolox Eq/L) (Erel, 2004; Erel 2005) by commercial kits using ELISA method (Table 1). Oxidative stress index (OSI) was determined by the ratio of the values of TOS to TAS (Lee and Kim, 2014). The results were reported as arbitrary units (AU).

Table 1: The sensitivity, coefficient of variations (intra- and interassay) and commercial provider of test kits for cortisol, total oxidant status (TOS) and total antioxidant status (TAS).

Test	Sensitivity	Coefficient of variations		Provider
		Intraassay	Interassay	
Cortisol	2.50 ng/mL	8.1%	6.6%	EIA – 1887, DRG, USA
TOS	1.20 mmol/L	3.9%	3.2%	Mega Tip, Gaziantep, Turkey
TAS	4.00 μmol/L	3.3%	2.8%	Mega Tip, Gaziantep, Turkey

Evaluation of Pain Scores

Modified Melbourne Pain Scale (MMPS) (Odette and Lesley, 2013) was used for the evaluation of pain by the same person, who did not know the groups in the study. This blind assessment was performed at the end of surgery (T0), 30 minutes after surgery (T^{1/2}) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery. Butorphanol (intravenous, 0.2 mg/kg, Butomidol, Richter Pharma, Austria) was used as a rescue analgesic, when the MMPS was scored higher than 9 points.

Statistics

The distribution of normality of data were analysed by Shapiro-Wilk normality test. All data had the normal distribution. Therefore, differences in total surgery time, length of skin incision, concentrations of cortisol, TOS and TAS, OSI rates and pain scores between groups were compared using the analysis of variance followed by t test. A repeated measures two-way ANOVA test was used to compare differences within the groups (SPSS 16.0). Data of total surgery time, length of skin incision and pain scores were described by mean ± Standard Deviation (SD), whereas cortisol, TOS, TAS and OSI were shown by mean ± Standard Error Mean (SEM). The data were considered to be significantly different at p < 0.05.

RESULTS

Final year veterinary students in the control group did not request any hands-on support during surgeries. Moreover, no complications occurred and no rescue analgesics were needed in the control and experiment groups throughout the study. It was observed that the total surgery time was 22.50 ± 4.50 minutes in the control group, as opposed to 58.50 ± 6.28 minutes in the experiment group ($p < 0.001$). The length of skin incision in the control group (1.88 ± 0.17 cm) was shorter ($p < 0.001$) than that detected in the experiment group (10.75 ± 1.40 cm).

The mean concentrations of cortisol in the control and experiment groups are given in Table 2. Accordingly, it was seen that the concentrations of cortisol at T-24 in the control group were lower than those at premedication time (T) but there was no statistical difference. On the other hand, the concentrations of cortisol were seen to be increased ($p < 0.001$) at the end of surgery (T0) and higher ($p < 0.001$) concentrations of cortisol were evident at T^{1/2}, T1 and T2 as compared to T-24 and T. It was observed that the concentrations of cortisol fell to baseline at T4 and T6 ($p < 0.001$). In the experiment group, a different cortisol concentration pattern was detected. The concentrations of cortisol at T showed nonsignificant increments as compared to T-24. However, it was

detected that the cortisol concentrations measured at the end of surgery (T0) and throughout T^{1/2}, T1, T2 and T4 were higher than those measured at T-24 and T ($p < 0.001$). The concentrations of cortisol at T6 were similar to the concentrations observed at T, T2 and T4, whereas the levels at T6 differed from those at other measurement times ($p < 0.001$). The comparison of cortisol concentrations between the control and experiment groups showed that there was no significant difference at T-24. Nevertheless, the cortisol concentrations at other measurement times in the experiment group were higher than those detected in the control group ($p < 0.001$) (Figure 1).

The mean concentrations of TOS, TAS and the OSI values in the control and experiment groups are given in Table 3. The concentrations of TOS and TAS in the control and experiment groups did not show any significant difference within and between groups (Figure 1). Similarly, OSI did not differ in the control and experiment groups. However, it was seen that OSI values at premedication time in the experiment group were higher than those calculated in the control group ($p < 0.05$).

Finally, pain assessment demonstrated that pain scores in both the control and experiment groups did not differ at any of the scoring times. Moreover, there was no significant difference between the control and experiment groups (Table 4).

Table 2: Blood cortisol concentrations (ng/mL) measured at 24-hour prior to surgery (T24), during premedication (T), at the end of surgery (T0), 30 minutes after the end of surgery (T^{1/2}) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after surgery in the control and experiment groups (Mean \pm SEM).

Blood sampling time (hour)	Control group (n = 12)	Experiment group (n = 12)
T-24	55.05 \pm 10.52 ^b	56.91 \pm 7.34 ^d
T	36.87 \pm 3.92 ^b	105.42 \pm 16.90 ^{cd}
T0	98.41 \pm 6.84 ^a	316.92 \pm 13.31 ^a
T ^{1/2}	106.68 \pm 7.85 ^a	300.58 \pm 12.67 ^a
T1	126.66 \pm 10.87 ^a	284.42 \pm 20.82 ^a
T2	100.62 \pm 10.10 ^a	257.60 \pm 29.66 ^{ab}
T4	59.89 \pm 7.95 ^b	168.48 \pm 24.92 ^{ab}
T6	46.81 \pm 5.57 ^b	138.07 \pm 7.98 ^{bc}

Superscript letters ^(abcd) indicate significant difference within groups ($p < 0.001$).

Table 3: Blood total oxidant status (TOS, $\mu\text{mol H}_2\text{O}_2$ Eq/L), total antioxidant status (TAS, mmol Trolox Eq/L) and the oxidative stress index (OSI, arbitrary unit) [TOS ($\mu\text{mol H}_2\text{O}_2$ Eq/L)/TAS (mmol Trolox Eq/L)] detected at 24-

hour prior to surgery (T24), during premedication (T), at the end of surgery (T0), 30 minutes after surgery (T½) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery in the control (C; n = 12) and experiment (E; n = 12) groups (Mean ± SEM).

Test	Groups	T-24	T	T0	T½	T1	T2	T4	T6
TOS	C	3.35±0.10	4.02±0.24	3.38±0.16	3.85±0.27	3.68±0.32	3.89±0.33	4.13±0.48	3.89±0.29
	E	3.22±0.22	4.08±0.36	3.56±0.28	3.20±0.10	3.84±0.24	3.76±0.37	3.95±0.32	3.85±0.37
TAS	C	1.81±0.10	1.74±0.10	1.70±0.15	1.70±0.19	1.59±0.24	1.78±0.21	1.79±0.03	1.79±0.04
	E	1.80±0.01	1.66±0.16	1.71±0.21	1.57±0.26	1.64±0.32	1.78±0.26	1.65±0.23	1.80±0.01
OSI	C	1.85±0.06	1.79±0.12*	2.30±0.14	2.48±0.24	2.01±0.18	2.08±0.11	2.26±0.13	2.09±0.18
	E	2.37±0.27	2.41±0.22	2.18±0.22	2.11±0.20	2.30±0.26	2.48±0.34	2.16±0.16	2.12±0.20

* Indicates significant difference between groups (p < 0.05).

Table 4. Distribution of pain scores (Mean ± SEM) detected at 24-hour prior to surgery (T24), during premedication (T), at the end of surgery (T0), 30 minutes after surgery (T½) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after end of surgery in the control and experiment groups (Mean ± SD).

Pain assessment time	Control group	Experiment group
(hour)	(n = 12)	(n = 12)
T0	3.00 ± 0.89	3.66 ± 0.81
T½	2.66 ± 0.51	2.83 ± 1.60
T1	2.56 ± 1.53	3.10 ± 0.51
T2	3.00 ± 0.63	2.66 ± 0.51
T4	2.83 ± 0.40	2.16 ± 0.75
T6	3.33 ± 1.63	2.50 ± 0.54

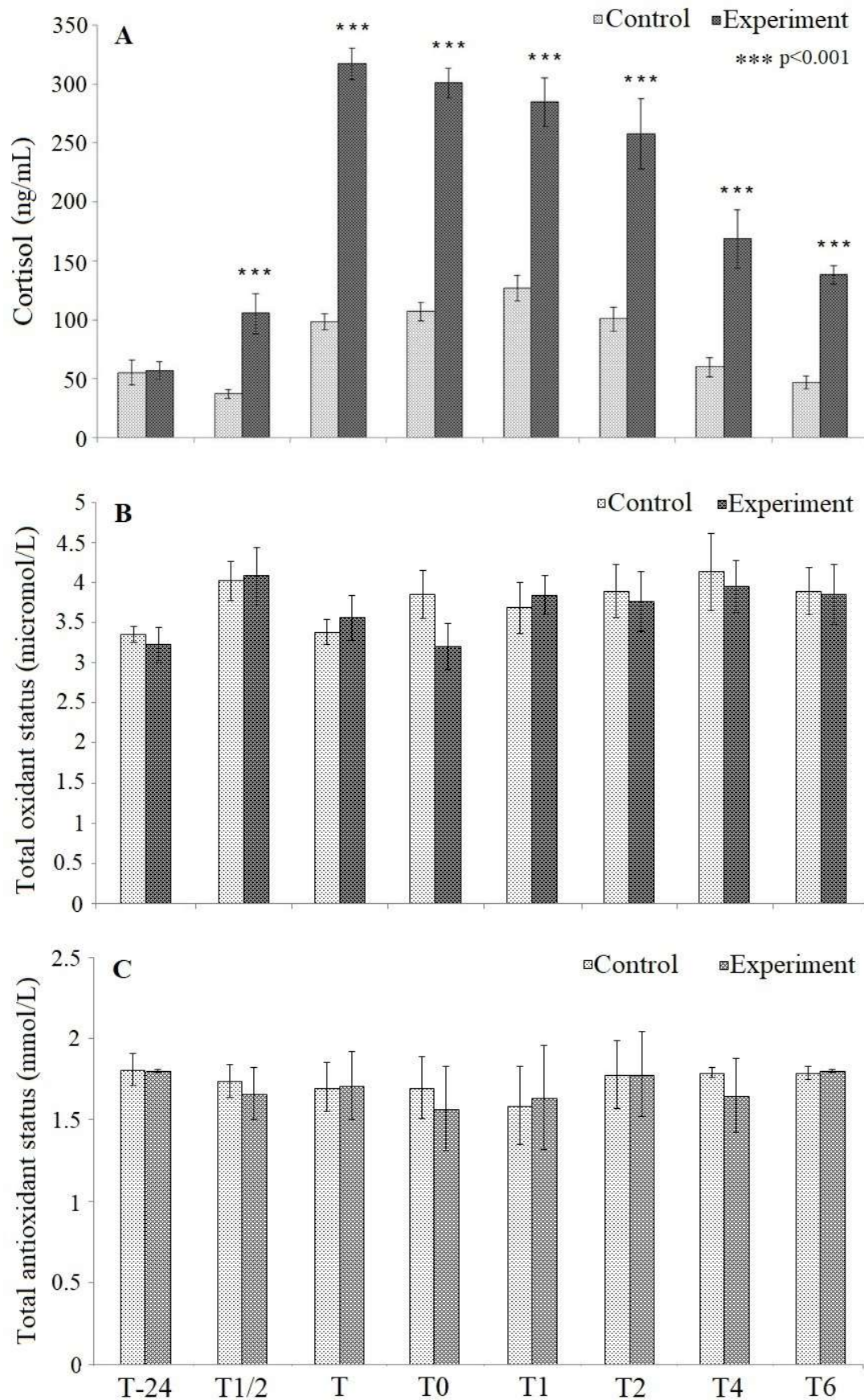


Figure 1. The comparison of blood cortisol (ng/mL) (A), total oxidant status ($\mu\text{mol H}_2\text{O}_2$ Eq/L) (B), total antioxidant status (mmol Trolox Eq/L) (C) at 24-hour prior to surgery (T24), during premedication (T), at the end of surgery (T0), 30 minutes after surgery (T $\frac{1}{2}$) and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery between the control and experiment groups.

DISCUSSION

Ovariohysterectomy, a common surgical procedure in dogs, is an elective surgery which causes tissue trauma, inflammation and pain due to intraabdominal surgical manipulations (Lemke et al., 2002). An elective surgery is performed on healthy animals which do not have any pre-existing pain. It is in evidence that OVH is a common model for evaluating postoperative stress and pain (Benson et al., 2000; Devitt et al., 2005; Michelsen et al., 2012; Tsai et al., 2013, Yilmaz et al., 2014, Korkmaz et al., 2019, Çolak and Yilmaz, 2020). In addition to this, small animal OVH as a live-animal surgery is the most common surgical procedure in veterinary teaching hospitals because the procedure contains basic knowledge about both anaesthesia and surgery. Therefore, the OVH model was evaluated in this study.

It is known that the total surgery time of OVH performed by experienced veterinary surgeons is 17 minutes on average with 1.5 – 2.5 cm width skin incision (Michelsen et al., 2012; Korkmaz et al., 2019). In contrast when students perform OVH, the duration of surgery time varies between 60 and 135 minutes, associated with the level of the students (Zeugschmidt et al., 2016) and the nature and type of teaching procedures such as how surgery is supervised (Bowlit et al., 2011; Harris et al., 2013; Freeman et al., 2014; Gates et al., 2018) and whether training includes tools such as virtual reality (Hunt et al., 2020), video recordings and plastic spay simulator models (Read et al., 2016; Shaver et al., 2019; Annandale et al., 2020). Similarly, the length of skin incision varies between 4 – 9 cm (Freeman et al., 2014) and 9.6 ± 3.4 cm (Harris et al., 2013). In the present study, it was observed that total surgery time (58.50 ± 6.28 minutes) and length of skin incision (10.75 ± 1.40 cm) in the experiment group was consistent with the above mentioned studies. The surgeries in the control group were performed in 22.50 ± 4.50 minutes with 1.88 ± 0.17 cm skin incision. It is suggested that the experience of the surgeon is inversely proportional to the total surgery time and the length of skin incision.

The concentrations of catecholamines, adrenocorticotrophic hormone (ACTH), cortisol and insulin increase in the body in response to surgical manipulations. The enhancement of these hormones continues to increase during surgery and reaches their peak concentrations at the end of surgery. The stress related to OVH is short term and it returns to preoperative values five hours after surgery (Benson et al., 2000) but the concentration of cortisol reaches its basal values 12 hours (Yilmaz et al., 2014; Korkmaz et al., 2019) or 24 hours after surgery (Church et al., 1994; Fox et al., 1994; Benson et al., 2000). It appears that the measurement of cortisol is a good indicator to evaluate stress response in dogs. Therefore, the concentrations of cortisol were evaluated in this study.

It was observed that the concentrations of cortisol, which started to increase at the end of surgery (T0) in the control group, returned to the premedication concentrations four hours after the end of surgery. The increasing concentrations of cortisol in the experiment group returned to premedication concentrations six hours after the end of surgery. It was indicated that this pattern observed in both groups was in accordance with other reports (Church et al., 1994; Fox et al., 1994; Benson et al., 2000; Kim et al., 2012; Yilmaz et al., 2014; Korkmaz et al., 2019). It is suggested that the reason for the late response of cortisol in the experiment group might be increased surgical trauma due to the longer total surgery time and length of skin incision. On the other hand, it was seen that the concentrations of cortisol measured at 24 hours prior to surgery did not differ significantly between the control and experiment groups, whereas the cortisol levels detected at other measurement times in the experiment group were higher than those observed in the control group. This observation supports that surgery time, the length of skin incision and the relevant experience of the surgeon may be the main factors affecting the concentrations of cortisol in blood after ovariohysterectomy.

Although one of the goals of this study was the investigation of the effect of surgery on pain response, the injection of any pain killer such as meloxicam was mandatory in both the control and the experiment groups due to ethical concerns. In doing so, it might be expected that the concentrations of cortisol were decreased by the injection of meloxicam via the inhibition of prostaglandins due to the inhibition of cyclooxygenase (COX) enzyme (Yilmaz et al., 2014). Meloxicam is a selective COX2 inhibitor and it inhibits the release of prostaglandins in the middle or long term (Distel et al., 1996). Moreover, it has been reported that the concentrations of cortisol remain high after OVH in dogs following the injection of meloxicam (Yilmaz et al., 2014). Therefore, it is suggested that the injection of COX1 selective anti-inflammatory drugs for acute response (Dow et al., 1990) may be an alternative to COX2 inhibitors such as meloxicam.

Oxidative stress in the body defines the production of reactive oxygen species (ROS) and the balance between ROS and the antioxidant defense mechanism to detoxify the ROS (Sies, 1997). The dense oxidative stress during surgery resulting in severe cellular damage may cause poor postoperative outcomes (Sies, 1997) thus, the minimisation of oxidative stress becomes crucial (Lee and Kim, 2014). On the other hand, the multiple actions of various antioxidants in the blood protect the organs against the harmful effects of ROS (Erel, 2004). The measurement of the concentrations of TOS and TAS in blood indirectly reflects the overall oxidative stress and antioxidant activity, respectively (Erel, 2004). Any unfavourable alteration in normal body homeostasis due to surgery is called surgical stress (Anup and Balasubramanian, 2000; Anup et al.,

1999). Therefore, if the surgery itself is involved in the surgical stress, an alteration of the concentrations of TOS would be expected after induction of anaesthesia and surgery (Koksal and Kurban, 2010; Lee and Kim, 2014). Nevertheless, in this study, the TOS concentrations within and between groups did not differ significantly which was an unexpected result. It has been reported that the changes in TOS are significantly higher in open surgery as compared to laparoscopic surgery in dogs (Lee and Kim, 2014). Therefore, it is suggested that open surgery itself, regardless of the length of surgery time, may induce oxidative stress, since open surgery has been performed in both groups in this study. Further studies with numerous samples are needed to clarify this interaction. Similarly, nonsignificant changes in TAS concentrations within and between groups were observed in the present study. Indeed, it has been reported that TAS levels are the same in humans undergoing hysterectomies whether the peritoneum is sutured or not (Szymczyk et al., 2003), hence, the type of surgery, surgical techniques or operation times may not cause any significant changes in TAS concentrations (Lee and Kim, 2014). It is suggested that the antioxidant response of the body does not change under surgical circumstances. The present study also evaluated the ratio of TOS to TAS to indicate the degree of oxidative stress. It was observed that the highest OSI values in the control and experiment groups were at T_{1/2} and T₂, respectively, but these changes were not significant. It has been stated that surgery on abdominal walls and the manipulation of the intestines leads to oxidative stress in enterocytes and this alteration remains stable for 24 hours (Anup et al., 1999). The oxidant/antioxidant status is related to the surgery technique (laparoscopic or open surgery) and surgery time (Anup et al., 1999; Kozlik et al., 2015). Although OSI values calculated at premedication time in the experiment group were significantly higher than those detected in the control group, OSI in other measurement times did not differ between groups. All the above mentioned findings may suggest that open surgery, regardless of the relevant experience of the surgeon and the surgery time, causes oxidative stress, and the oxidative stress in ovariohysterectomies is not increased in surgical procedures performed by final year veterinary medicine students.

The attitudes of veterinary practitioners towards pain management reveals a lack of adequate awareness and knowledge of pain assessment, analgesics, and pain recognition and quantification in assessing the effectiveness of pain treatment (Raekallio et al., 2003; Hugonnard et al., 2004; Williams et al., 2005; Hewson et al., 2006). Several types of pain scoring systems have been developed to grade the severity of postoperative pain in animals. Pain scoring is based on the numerical rating of various indicators and behaviours and the total value is used for the assessment of severity of pain (Mathews et al., 2014). It seems that the modified

Melbourne Pain Scale is the more sensitive and specific pain assessment procedure in veterinary medicine (Firth and Haldane, 1999). It is known that the anaesthesia, surgery time, length of skin incision and the experience of the surgeon are the main factors affecting the postoperative pain scores (Carpenter et al., 2004; Devitt et al., 2005; Campagnol et al., 2012; Michelsen et al., 2012). Moreover, the possible surgery-related complications of performing OVH include intraabdominal haemorrhage, vaginal bleeding and ligation of the ureter in the short term (Van Goethem et al., 2006). Contrary to expectations in the present study, Modified Melbourne Pain Scoring showed that there was no significant difference within and between groups without the presence of any surgical complications. Although total surgery time was longer in the experiment group, it is suggested that the basic knowledge of final year veterinary medical students is sufficient to avoid undue trauma, when performing OVH and thereby causing no unnecessary severe pain. In conclusion, it is indicated that blood cortisol concentrations tend to increase in dogs undergoing OVH performed by final year veterinary medical students relating to the surgery time and the length of skin incision. It is nevertheless possible to conclude that OVH may be safely performed by veterinary medical students under the supervision of an experienced veterinary surgeon in dogs without any postoperative pain-related behavioural changes. Moreover, it is believed that supervision by employers of newly graduated veterinary surgeons might be beneficial in their employment to enhance their judgement and decision-making skills.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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