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RESEARCH ARTICLE

Comparison of Acute Phase and Haemostatic Responses Following Ovariohysterectomy via Midline or Flank Approach in Dogs

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A B S T R A C T

Different contraception techniques have been recommended in dogs for population control, behavioral management, and health benefits. However, ovariohysterectomy (OVH) is still the most widely used technique in veterinary practice. This study aimed to evaluate haematobiochemical changes and surgical trauma in ventral midline and right flank laparotomy approach for assessing the effectiveness of OVH techniques commonly used in dogs. Clinically healthy non-pregnant dogs (n=20, 1-5 years of age with 22.3±1.5kg mean BW) were randomly divided into two groups to perform OVH via ventral midline (n=10) or right flank laparotomy (n=10). The surgical time, length of the surgical incision, and time taken by the dog to recover from anesthesia were evaluated to compare surgical techniques. The haematobiochemical changes and surgical trauma were determined by taking blood samples before surgery and at specific intervals afterward by measuring haematological parameters, C-reactive protein (CRP), fibrinogen, and D-dimer values. According to the results, a statistically non-significant difference was observed in serum CRP levels between the two groups. A significant reduction ($P<0.05$) in platelet count was observed in both groups on the post-operative day-2 compared to pre-operative or post-operative day-7 values. While there was significant difference in fibrinogen values between preoperative and 12h postoperative measurements ($P<0.05$), there was no difference between the two groups. Although the preoperative serum D-dimer values could not be evaluated statistically, no difference was found between times and groups in the postoperative serum D-dimer values at 6 and 12h. In conclusion, the results demonstrated that ovariohysterectomy performed by ventral midline and right flank laparotomy in dogs had a similar haemostatic response, and that both approaches can be safely preferred.

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INTRODUCTION

Different contraception techniques have been recommended to prevent overpopulation or unwanted reproductive disorders in female dogs. Even though non-surgical contraception methods such as the use of hormones or chemicals have been described (Rafatmah *et al.*, 2019; Karadağ *et al.*, 2024), the surgical contraception technique of ovariohysterectomy (OVH) is still the most widely used technique in veterinary practice (Zink *et al.*, 2023). Ovariohysterectomy can be performed through ventral midline (midline-OVH) or right flank laparotomy (flank-OVH) in dogs (Howe, 2006; Karakaya-Bilen *et al.*, 2023). It is well known that any surgical approach creates a series of metabolic, hormonal, and inflammatory reactions due to surgical stress and trauma in the body (Rodigheri *et al.*, 2023). Therefore, there is great interest in selecting minimally invasive methods for OVH, which may reduce surgical stress, tissue trauma and have less postoperative complications (Lu *et al.*, 2016; Hernández-Avalos *et al.*, 2021). Over the years, lateral flank laparotomy approach has been extensively discussed for spaying female cats. However, only a few studies have compared midline-OVH and flank-OVH in dogs (Acharya *et al.*, 2016; Dhakal *et al.*, 2023), and to the best of our knowledge, no studies have examined differences in the postoperative surgical trauma and the haemostatic component of the surgical stress response between the two procedures. While the midline-OVH presents challenges such as wound dehiscense, evisceration and herniation (Howe, 2006), the flank-OVH may offer distinct benefits in cases of excessive mammary development or when postoperative examination is likely to be limited (Yun *et al.*, 2021). Although no comprehensive study has been conducted on this topic in dogs, it is thought that midline OVH minimizes vascular damage by causing less bleeding due to anatomical location of the surgical site (Howe, 2006).

The acute phase response (APR) is a non-specific reaction to tissue disturbances such as surgery, trauma, infection, or neoplasia, which can disrupt homeostasis; and magnitude of the latter increases in proportion to the extent of surgical trauma (Christensen *et al.*, 2015). C-reactive protein (CRP) is a positive acute-phase protein and a crucial biomarker for tracking inflammation in dogs, offering greater precision and reliability in detecting systemic inflammation after surgery compared to conventional indicators such as body temperature and leukocyte levels (Malin and Witkowska-Piłaszewicz, 2022). Regularly assessing its levels may facilitate a more accurate assessment of postoperative inflammatory reaction throughout the recovery period (Christensen *et al.*, 2015; Karakaya-Bilen, 2019).

Haemostasis is a critical physiological process that ensures proper blood clotting while preventing excessive bleeding or thrombosis, and in dogs, some hemostasis parameters are commonly used to assess coagulation status and diagnose potential bleeding disorders (Schwarz *et al.*, 2021). Fibrinogen, D-dimer levels, and platelet count (PLT) are key parameters in assessing hemostasis. Fibrinogen, the primary precursor of fibrin which is a fibrous, non-globular protein involved in the clotting of blood, reflects ability of the body to generate stable clots. D-dimer levels serve as a crucial biomarker for detecting fibrinolysis and thromboembolic conditions, indicating excessive clot breakdown. Platelet count plays a fundamental role in primary hemostasis, as platelets are essential for clot formation and wound healing. These parameters are widely used in clinical and research settings to evaluate coagulation status, monitor post-surgical recovery, and diagnose haemostatic disorders (Moldal *et al.*, 2012; Shipov *et al.*, 2018; Antunes *et al.*, 2024).

Surgical procedures induce physiological and haematobiochemical changes, primarily due to the stress response triggered by tissue trauma. Minimizing surgical stress through the selection of an appropriate surgical approach is crucial for ensuring the well-being of the patient. The objective of this study was to compare the acute phase response and haemostatic parameters by examining CRP, PLT, fibrinogen and D-dimer values in ovariohysterectomy operations performed with midline-OVH and flank-OVH in healthy dogs. By evaluating these parameters, this study aimed to identify the surgical sterilization technique that induces the least physiological disturbances and minimizes invasiveness, thereby determining the safest surgical sterilization technique in dogs.

MATERIALS AND METHODS

Animals: In this study, a total of 20 clinically healthy female dogs of various breeds with the age of 1-5 years and a mean body weight of 22.3±1.5kg, were used. These dogs were brought to Siirt University Animal Health Application and Research Centre for OVH during the period from April to July, 2022. Dogs that were too aggressive, pregnant or had any health problem were not included in the study. Because the dogs in the study weighed 20kg or more, they were considered to have a wide body conformation. The animals included in the study had their body weight and height at the withers measured before OVH. The animal owners were informed about the study before the operation and their written consent was obtained. Preoperative routine health checks (body temperature, pulsation and respiration rate, lymph nodes palpation, tracheal palpation, lung auscultation) were carried out. Based on the findings of vaginal cytology (Kustritz, 2020), the dogs were found to be in the anestrus phase. The animals that were deemed suitable for surgery were hospitalised for food and water restriction the night before the operation. The clinical part of the study was approved by Siirt University Animal Experiments Local Ethics Committee with the approval number 2020/04.

Experimental design and ovariohysterectomy: The dogs were randomly divided into two groups for midline-OVH (n=10) and flank-OVH (n=10). The duration of the operation (including the time taken to make the incision, perform the procedure, and place the final skin sutures), the length of the incision measured with calipers, as well as the time taken by the dog to wake up from anesthesia, were recorded. Body temperature, respiration rate, and pulse rate were recorded preoperatively, as well as at the 6th and 12th hour, 1st, 2nd and 7th day postoperatively.

Before surgery, induction of the general anesthesia was established with 2.3mg/kg BW xylazine HCl (Xylazinbio 2%, Bioveta®, Czech Republic), followed by 10mg/kg BW ketamine HCl (Ketasol 10%, Richter Pharma-Interhas®, Turkey). During the surgical procedure, all experimental dogs received fluid therapy through the cephalic vein using a saline solution (10mL/kg/h). The dogs in the midline-OVH group were placed on the table in the ventro-dorsal position and the surgical area was prepared for aseptic surgery. A straight incision was made 2 fingers below the umbilical scar. After entering the abdominal cavity through the linea alba, a routine ovariohysterectomy was performed (Karakaya-Bilen, 2019). The abdominal muscles and peritoneum were sutured together using absorbable suture material (PGLA® UPS 0, Katsan Inc. İzmir, Turkey)) with a cross (X) mattress suture technique. The skin was sutured (PGLA® UPS 0, Katsan Inc. İzmir, Turkey) using horizontal mattress sutures technique.

Dogs in the flank-OVH group were prepared for the aseptic surgery in the right paralumbal fossa in the region between the last rib, tuber coxae, and the vertebral column. The skin was incised through vertical right flank approach and the routine OVH procedure was then completed (Karakaya-Bilen *et al.,* 2023). To ensure uniformity in the experimental design, all OVH operations were carried out by the same experienced surgeon with the help of an assistant. All animals received analgesic (Demelox®, meloxicam 0.2mg/kg, Denova®, Turkey) preoperatively and prophylactic antibiotic (Cefatek 15%®, cephalexin monohydrate 10mg/kg/day, Teknovet®, Turkey) was given daily for five days postoperatively. No postoperative complications were observed in any of the animals.

Blood sampling and performing analyses: To obtain complete blood cell counts and biochemistry profiles, blood samples were collected from all dogs via the cephalic vein into serum tubes without anticoagulant and into a tube containing EDTA (BD Vacutainer®, New Jersey, USA) as an anticoagulant. Blood samples were collected before surgery and then at 6 and 12h, and 1, 2 and 7 days after operation. Following collection, blood samples were held at room temperature for 30-45 minutes, and subjected to centrifugation at 3500 rpm for 15 minutes. The plasma and serum, which formed the top layer in the tube, were carefully transferred into a microsantrifuge tube and stored at -20°C for analysis.

Haematological parameters and PLT counts were determined preoperatively, and on the 2nd and 7th day postoperative, using a fully automatic veterinary blood counting device (MINDRAY BC-2800Vet, Blood Counting Device, China). A high-sensitivity CRP (hs-CRP) was measured preoperatively, as well as at 6 and 12 hours, and 1, 2, and 7 days postoperatively. The CRP analyses were performed using canine high sensitivity C-Reactive protein ELISA Kit (BT Lab, China; Cat. No E0130Ca). According to the data provided by the manufacturer, the intra-assay and inter-assay coefficients of variation (CV) for the kit were <8 and <10%, respectively. Serum fibrinogen levels were measured preoperatively and at 6, 12, and 24 hours postoperatively using a canine fibrinogen ELISA kit (BT Lab, China; Cat. No E0126Ca). Intra- and interassay CV were 1.2 to 7.8%. Serum D-dimer levels were measured preoperatively and at 6 and 12 hours postoperatively using a Fluorescent Immunoassay rapid test (Finecare, Wondfo Biotech Co. Ltd, Finecare, Atateknik, Turkey) with FIA meter commercial test kits (D-Dimer test, Finecare, Wondfo Biotech Co. Ltd).

Statistical analysis: To summarize the data and determine whether the assumptions were true, descriptive analysis was utilized, along with Kolmogorov–Smirnov for normality, Levene's test for homogeneity of variances, and Mauchly's test for sphericity. Regarding sphericity violation, Greenhouse-Geisser correction was applied. A two-way repeated measures analysis of variance was performed to determine differences in different variables between the two experimental groups and time points (Maxwell and Delaney, 2004). The median and lateral factors were identified as between-groups variables, whereas the 6^{th} and 12^{th} hour, 1^{st}, 2^{nd} and 7^{th} day (where applicable) were determined as within-group variables. Data on body weight, withers height, incision size, operation time, awakening time and complete blood count data were analysed using the Independent Sample T-Test. CRP and fibrinogen levels were analyzed by two-way repeated measures analysis. All data were analysed using SPSS 26 (Chicago, Illinois, USA, SPSS Inc.). A P-value of <0.05 was deemed significant for all analyses. The variable D-dimer concentration was not normally distributed and was logarithmically transformed prior for inclusion in the linear mixed model.

RESULTS

There were non-significant differences among animals of the two OVH groups in terms of body weight, withers height, incision length, operation time, and recovery time from anaesthesia (Table 1). A comparison of the two approaches revealed statistically significant differences in body temperature (Table 2), with midline-OVH consistently exhibiting higher values than flank-OVH across all time points (main group effect: P<0.05). When the time factor was considered, it was found that body temperature and pulse rate values decreased significantly on postoperative day-7 compared to pre-operative values (P<0.01). However, respiration rate varied non-significantly among time points and OVH groups. Similarly, variations in body temperature, respiration rate and pulse rate due to Time X OVH group interaction were non significant (Table 2).

Haematological parameters were close to the reference values in both groups preoperatively. There were non-significant differences between midline-OVH and flank-OVH groups on postoperative day-2 and day-7 in all haematological parameters, with the exception of haemoglobin (HGB). On postoperative day-7, a statistically significant difference was found between the groups in HGB value, and its level was higher in midline-OVH than flank-OVH group (P<0.05, Table 3).

There were non-significant differences between the groups in preoperative or postoperative CRP levels. While the preoperative serum CRP level was at basal level before operation, it increased significantly (P<0.05) at the 6th and 12th hours postoperatively, reaching the highest level on postoperative day-1, followed by a significant decline on postoperative days 2 and 7 (Fig. 1). The PLT counts did not differ significantly between the two OVH groups at any time point but these were significantly lower (P<0.01) at 2 days after surgery compared to their value pre-operation or 7 days after surgery (Fig. 2). Similarly, non-significant differences were observed in serum fibrinojen levels between the two surgical approaches at all time points, however, statistically significant differences were recorded among time points (P<0.05), being the highest 12h after operation and lowest at 1 day after operation (Fig. 3). As serum D-dimer values were within the normal range prior to the operation, they could not be statistically evaluated. Results from the 6th and 12th hours after the operation showed non-significant differences in serum D-dimer values between groups and between times (Table 4). Similarly, variation in D-dimer values due to Time X Group interaction was statistically non-significant.

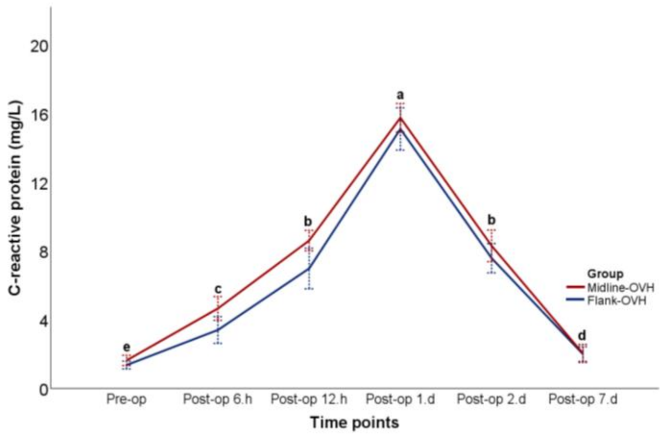

Fig. 1: Changes in serum C-reactive protein (CRP) levels (mg/L) over time in dogs undergoing midline-OVH or flank-OVH. Different letters indicate statistically significant differences between time points (P<0.05). Error bars represent standard errors.

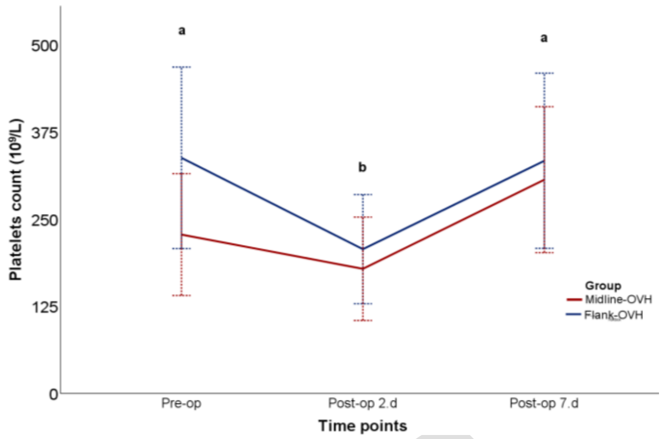

Fig. 2: Changes in platelet (PLT) count (10^9/L) over time in dogs undergoing Midline-OVH or Flank-OVH; Different letters indicate statistically significant differences between time points (P<0.05).

Table 1: Variables (mean±SE) recorded in Midline-OVH and Flank-OVH groups

Variables	Group	Mean±SE	Median (Q_1-Q_3)	Min.	Max.	P-value
Body weight (kg)	Midline-OVH	23.57±2.00	23.50 (22.60-25.00)	9.45	35	0.435
	Flank-OVH	21.07±2.50	19.05 (15.66-26.51)	11.80	36	
Withers height (cm)	Midline-OVH	55.95±2.10	56.25 (55-59.50)	39.00	63	0.324
	Flank-OVH	54.50±1.50	55.00 (50.50-56.75)	48.00	62	
Incision length (cm)	Midline-OVH	2.89±0.30	3.00 (1.85-3.38)	1.80	5	0.939
	Flank-OVH	3.15±0.50	2.55 (2.00-3.88)	1.50	7	
Operation time (min.)	Midline-OVH	15.50±1.20	14.00 (13.00-16.50)	11.00	22	0.789
	Flank-OVH	17.50±3.00	13.00 (12.00-18.00)	10.00	36	
Recovery time from anesthesia (min)	Midline-OVH	57.90±4.80	61.00 (49.00-64.75)	31.00	87	0.453
	Flank-OVH	62.80±4.20	61.50 (54.25-73.75)	43.00	80	

Table 2: Changes in vital parameters depending on time and OVH approaches (mean±SE)

Parameters	Group	Pre-op	Post-op 6.h	Post-op 12.h	Post-op 1.d	Post-op 2.d	Post-op 7.d	P-value		
								Time	Group	Time x Group
Body temperature (°C)	Midline-OVH	$39.0±0.42^{A, a}$	$38.9±0.60^{A, a}$	$38.9±0.56^{A, ab}$	$38.5±0.52^{A, ab}$	$38.5±0.47^{A, ab}$	$38.3±0.55^{A, b}$	<0.001	0.02	0.62
	Flank-OVH	$38.5±0.69^{B, a}$	$38.5±0.53^{B, a}$	$38.3±0.41^{B, ab}$	$38.1±0.42^{B, ab}$	$38.2±0.52^{B, ab}$	$38.1±0.45^{B,b}$			
Pulsation rate (BPM)	Midline-OVH	$112.0±16.40^{a}$	$118.0±26.90^{ab}$	$115.0±21.90^{a}$	$106.0±18.20^{a}$	$101.0±17.20^{ab}$	$93.2±17.70^{b}$	<0.001	0.80	0.60
	Flank-OVH	$108.0±18.70^{a}$	$106.0±22.80^{ab}$	$110.0±26.50^{a}$	$106.0±13.40^{a}$	$99.8±13.70^{ab}$	$94.8±13.90^{b}$			
Respiratory rate (/min)	Midline-OVH	33.8±6.56	32.0±5.97	34.8±12.50	30.8±7.32	33.0±8.96	30.4±6.02	0.055	0.69	0.10
	Flank-OVH	32.0±9.00	26.4±6.31	27.4±9.34	27.1±5.38	29.6±5.80	25.2±6.68			

Values with different lowercase superscripts indicate statistically significant differences between time points within the same group. Values with uppercase superscripts denote significant differences between groups (P<0.05). BPM: Beats per minute.

Table 3: Changes in hematological parameters by time and OVH approaches

Hematological parameters	Group	Pre-op		Post-op 2.d		Post-op 7.d	
		mean±SE	P	mean±SE	P	mean±SE	P
WBC (10^9/L)	Midline-OVH	11.10±0.9	0.15	16.04±1.1	0.27	11.86±0.9	0.34
	Flank-OVH	13.03±0.8		18.86±2.4		11.85±1.5	
Lenf. (10^9/L)	Midline-OVH	1.59±0.3	0.70	3.73±0.5	0.89	1.99±0.3	0.33
	Flank-OVH	1.79±0.3		3.85±0.6		1.63±0.4	
Mon. (10^9/L)	Midline-OVH	1.64±1.1	0.21	0.71±0.06	0.08	0.47±0.05	0.28
	Flank-OVH	0.48±0.06		1.28±0.2		0.62±0.1	
Gran. (10^9/L)	Midline-OVH	8.70±1.4	0.14	12.9±0.76	0.11	9.13±0.5	0.31
	Flank-OVH	11.41±1.02		15.10±1.57		9.54±1.2	
Eos. (%)	Midline-OVH	3.76±1.5	0.58	3.82±0.71	0.31	2.33±0.4	0.11
	Flank-OVH	2.98±0.3		2.87±0.57		1.54±0.2	
RBC (10^12/L)	Midline-OVH	6.88±0.3	0.14	6.37±0.5	0.34	6.34±0.3	0.09
	Flank-OVH	6.24±0.3		5.87±0.2		5.45±0.4	
HGB (g/dl)	Midline-OVH	14.51±0.6	0.20	14.20±1.1	0.32	$14.62±0.6^{a}$	0.04
	Flank-OVH	13.32±0.6		12.96±0.6		$11.90±1.1^{b}$	
HCT (%)	Midline-OVH	45.82±2.07	0.19	45.06±3.3	0.29	43.61±1.7	0.07
	Flank-OVH	41.89±2.0		41.33±1.4		37.16±2.8	
MCV (fL)	Midline-OVH	67.60±0.8	0.76	70.86±0.7	0.79	68.92±0.6	0.58
	Flank-OVH	67.15±1.2		70.50±1.07		68.26±0.9	

Values with different superscripts indicate significant differences between groups (P<0.05).

Table 4: Changes in D-Dimer (ng/mL) levels by time and OVH approaches (mean±SE).

Group	Post-op 6.h	(min–max)	Post-op 12.h	(min–max)	P Value		
					Time	Group	Time x Group
Midline-OVH	233.15±36.2	137.2–310.0	296.51±109.7	108.4–554.8	0.23	0.16	0.83
Flank-OVH	123.32±7.5	108.1–154.3	212.27±62.8	105.9–468.1			

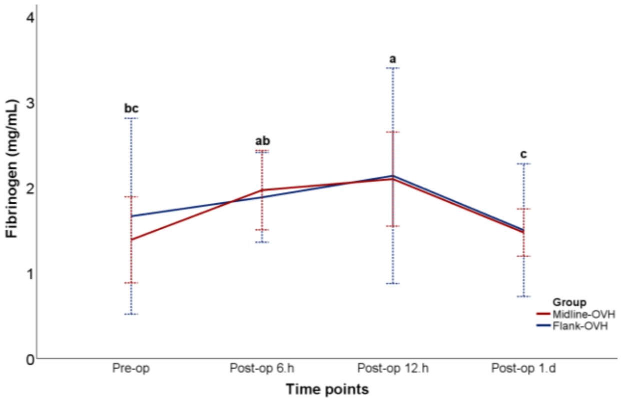

Fig: 3: Changes in serum fibrinogen (mg/mL) levels over time in dogs undergoing Midline-OVH or Flank-OVH; Different letters indicate statistically significant differences between time points (P<0.05).

DISCUSSION

Neutering is a common surgical procedure performed in dogs for population control, behavioral management, and health benefits. However, the choice of the surgical site like ventral midline, flank, or other approaches may influence physiological response of the animal, particularly in terms of acute phase reactions and hemostasis. Both midline and flank techniques have their respective advantages and limitations, influencing surgical outcomes, postoperative recovery, and complication rates (Arunkumar *et al.*, 2017; Karakaya-Bilen *et al*, 2023).

In some studies, it has been reported that morphometric data such as body weight and withers height have possible effects on parameters such as operation time, recovery time from anesthesia and incision length (Howe 2006; Muraro and White, 2014; Shaver *et al.*, 2019). Therefore, recording such morphometric data could have contributed to more accurate interpretation of the differences between surgical approaches. However, in this study, no differences were found between the midline-OVH and flank-OVH groups in terms of morphometric data such as body weight and withers height, operation time, recovery time from anesthesia and incision length.

In a study by Gates *et al.* (2020), 278 of 282 participants (98.6%) who performed canine ovariohysterectomy used the ventral midline approach, while only three participants used the lateral flank approach and one participant performed laparoscopic OVH in New Zealand. Similarly, the ventral midline approach is also predominantly used for canine ovariohysterectomy procedures in the United States and the United Kingdom (Howe, 2006). This preference is mainly due to the fact that the ventral midline approach is the primary technique taught in veterinary faculties (Gates *et al.*, 2020). However, it is important to emphasize that no specific technique has been proven superior to others, and many choices made in private practice are primarily driven by practical or financial considerations without affecting patient safety. The choice of surgical approach should be based on the surgeon's expertise, patient anatomy, and clinical circumstances (Shaver *et al.*, 2019). The right flank approach can serve as a viable alternative to the ventral midline approach in specific cases, particularly for reducing postoperative complications in stray or unmonitored dogs. This method provides direct access to the ipsilateral ovary and uterine horn, potentially simplifying their exteriorization (Mcgrath *et al.*, 2004; Karakaya-Bilen *et al.*, 2023). In addition, according to McGrath *et al.* (2004) and Arunkumar *et al.* (2017), the flank approach may present greater technical challenges than the ventral midline and may be unsuitable for dogs with wide body conformation or thick trunk musculature. Also, McGrath *et al.* (2004) noted that every dog with wide body conformation may not have a disadvantage; individual anatomy is important. According to Yun *et al.* (2021), lateral flank approach was successfully used to perform OVH on a lion with a ruptured pyometra. Although in our study, dogs weighing more than 20kg were considered to have a wide body conformation, there was no difference in operation time, recovery time and incision length between the two groups, and it was observed that the operator could access the right and left ovaries and uterus more easily through the flank approach. Morever, no complications were observed during the operation or postoperative period with both approaches.

Surgical wound healing is influenced by factors like incision placement, duration of operation, surgeon experience, and haemostatic control (Özaydın and Aydın, 2023). McGrath *et al.* (2004) reported that the flank incision may shorten surgical time and allow for smaller incisions because the ipsilateral ovary and uterine horn are located directly under the incision. Similarly, previous studies have reported that the right flank approach generally results in shorter incisions than middline approach, with flank incision lengths in the range of 1.0-4.8cm (Reece *et al.*, 2012; Sharda *et al.*, 2020; Dhakal *et al.*, 2023). However, in the present study, there was non-significant difference in mean incision length between the midline-OVH (2.89cm) and the flank-OVH group (3.15cm), and wound healing proceeded uneventfully in both cases.

General anesthesia can affect the regulation of body temperature, respiratory and pulsation rate (Rodriguez-Diaz *et al.*, 2023, Hofmeister, 2024). Hypothermia is reported as a common complication of general anesthesia and has been releated with longer recovery time from anesthesia (Pottie *et al.*, 2007). Despite the report by Wenham and Santos (2024), body temperature did not change from preoperative to postoperative day-2 in both surgical groups. However, a significant decrease was observed on postoperative day-7 compared to all previous time points (P<0.05). Despite all this, values remained within the physiologic range (37.5-39.2°C), suggesting no clinical hypothermia (Rodriguez-Diaz *et al.*, 2023). This decrease in body temperature on postoperative day-7 may be related to decreased physical activity, housing environment or postoperative metabolic adaptations. Similarly, in both groups, pulse rate did not differ between groups, but the change was related to time. The lowest mean pulse rate was recorded postoperative on day-7 in each surgical group. This decrease was thought to be related to the postoperative recovery process, decrease in stress and excretion of anesthetics from metabolism (Costa *et al.*, 2023; Hofmeister, 2024). In this study, although significant changes were observed in some vital parameters over time in the postoperative period, their values were within physiologic limits, indicating that both techniques were well tolerated by healthy dogs and did not cause any physiologic stress.

Previous studies have indicated that both surgical techniques have a similar impact on haemogram values. For instance, Arunkumar *et al.* (2017) observed slight changes in hematological and biochemical parameters in both groups. Similarly, Acharya *et al.* (2016) reported non-significant increases in WBC, neutrophils, monocytes, and fibrinogen levels, along with a decrease in packed cell volume due to surgical approaches. In our study, haematologic parameters (except HGB) were similar between the two surgical groups. In this study, only on postoperative day-7, a significant increase in HGB was noted in middline-OVH (14.62g/dL) compared to 11.90g/dL in flank-OVH group. This increase in HGB on postoperative day-7 may be related to the rearrangement of fluid balance in the body, delayed inflammatory responses during the healing process, or late hematologic responses due to differences between surgical approaches (Hofmeister, 2024).

The acute phase response, a key component of the innate immune system, is triggered by surgical trauma and marked by the release of acute phase proteins such as CRP. C-reactive protein can increase up to 95-fold postoperatively, correlating with the extent of tissue damage (Yamamoto *et al.*, 1993; Christensen *et al.*, 2015). Peak CRP levels typically occur within 12–24 hours post-surgery (Nevill *et al.,* 2010) and may remain elevated for 7 to 17 days, depending on the severity of trauma (Dabrowski *et al.,* 2007). As homeostasis is restored, CRP levels gradually decline (Dabrowski and Wawron, 2014). Higher CRP concentrations are often observed due to prolonged surgery times and increased tissue trauma (Michelsen *et al.*, 2012; Darounkolaei *et al.*, 2023). In the present study, all procedures were performed by an experienced surgeon, CRP levels increased similarly in both surgical groups on postoperative day-1 and gradually declined thereafter. An increase in CRP levels was expected within the first 24 hours after surgery as part of the physiological acute phase response to surgical trauma. This pattern suggests that both surgical techniques induced comparable levels of tissue trauma and that the resolution of inflammation and restoration of homeostasis followed an almost similar course in both groups.

Haemostatic balance is crucial for surgical outcomes, as surgical stress can affect coagulation pathways, fibrinolysis, and platelet function. The degree of haemostatic alterations varies with surgical site due to differences in vascularization, tissue trauma, and local inflammatory responses (Özaydın and Aydın, 2023). Various studies have demonstrated that elective surgeries, including ovariohysterectomy and ovariectomy, induce a haemostatic response characterized by a transient hypercoagulable state postoperatively (Moldal *et al.*, 2012). The surgical approach itself may also influence haemostatic response. Dhakal *et al.* (2023) reported that the right flank approach led to reduced bleeding and fewer wound complications compared to the ventral midline approach. The anatomical differences between the two approaches, particularly regarding the vascularity of the incision site, may play a crucial role in these outcomes. In this study, PLT counts remained comparable between groups, yet a significant decrease was noted on postoperative day-2, indicating temporary platelet consumption due to increased coagulation activity following surgical trauma (Millis *et al.,* 1992; Moldal *et al.,* 2012). Christensen *et al.,* (2015) also highlighted the impact of surgical stress on platelet function and counts. Fibrinogen, a key acute-phase reactant, shows a postoperative increase, supporting its role in compensatory clot formation and tissue healing (Ceron *et al.,* 2005). Fibrinogen levels did not differ between the groups, but exhibited temporal changes, with highest values 12h post-operative. This temporal alteration in fibrinogen levels underscores its function as a marker of surgical trauma and postoperative recovery, as evidenced by previous studies (Sobiech *et al.,* 2011: Moldal *et al.*, 2012). Additionally, D-dimer levels, a key marker of fibrinolysis and thrombus degradation, suggest a transient prothrombotic state followed by fibrinolysis, a pattern also observed in human and veterinary surgical models (Stokol, 2003; Moldal *et al.*, 2012). In this study, preoperative serum D-dimer levels were within the normal range and thus not statistically evaluated. Postoperative results at 6 and 12h showed non-significant differences between groups, compatible with Shipov *et al.* (2018), who reported stable D-dimer levels after soft tissue and orthopedic surgeries in dogs. Similar findings by Nelson and Andreasen (2003) suggest that surgical approach may not affect D-dimer levels in the immediate postoperative period. Interestingly, Ke *et al.* (2023) found elevated preoperative D-dimer levels in dogs undergoing tumourectomy due to tumour-induced coagulation activation, with a subsequent postoperative decrease. This highlights the distinct haemostatic responses in healthy versus neoplastic dogs undergoing surgery.

Conclusions: This study provides valuable insights into the comparative efficacy of ventral midline and right flank laparotomy approaches for ovariohysterectomy in dogs. The findings indicate that both surgical techniques induce comparable haematobiochemical responses, suggesting that neither approach has a distinct haemostatic advantage over the other. These results support the flexibility of surgical choice based on surgeon preference, patient anatomy, or clinical circumstances without compromising haemostatic stability or postoperative outcomes.

Authors contribution: EKB and GA designed experiments, and prepared the original manuscript; EKB, GA and MAK carried out experiments, analyzed blood samples and revised the manuscript; EG analyzed experimental results and edited the manuscript. All authors interpreted the data, critically reviewed the manuscript for important intellectual contents and approved the final version.

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