



# Comparison of Ventral Midline and Right Flank Approaches of Ovariohysterectomy in Bitches

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**Abstract** The ventral midline approach (VMA) and right flank approach (RFA) are common procedures for the sterilization of bitches. This study compared the different parameters viz. total duration of surgery, recovery time, and length of the incision as well as body temperature, heart rate, respiration rate, and SpO<sub>2</sub> in each approach. Twenty (20) bitches were divided randomly for the RFA and VMA. Meloxicam (0.2 mg/kg) was administered subcutaneously half an hour before the induction to provide preemptive analgesia. Diazepam and ketamine were administered intravenously at dose rates of 0.25 mg/kg and 2.5 mg/kg, and 0.17 mg/kg and 3.33 mg/kg, respectively to produce and maintain anesthesia. Each parameter was recorded at the pre-operative, operative and post-operative times. The average duration of surgery and length of incision of RFA (16.1 ± 5.13 min and 2.44 ± 0.83 cm) were significantly lower ( $p < 0.05$ ) than the VMA (21.3 ± 5.48 min and 3.53 ± 0.7 cm). The operated bitches showed hypothermia ( $p < 0.05$ ) at 1 hour compared to baseline and 24 hours of surgery. Heart and respiration rates increased significantly ( $p < 0.05$ ) during traction and severing of ovarian ligaments in bitches within the RFA group, but there was no significant difference within VMA approaches. The sedation score was significantly higher ( $p < 0.05$ ) at 1 hour after surgery in both approaches. Based on the duration of surgery and length of incision RFA approach was quick and minimal skin wound. Further studies on bitches considering molecular investigations of surgical stress are imperative.

**Key words** sterilization of bitches, ovariohysterectomy, ventral midline approach, right flank approach, surgery.

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## Introduction

In developing nations like Nepal, most dogs stray and wander freely, posing a severe threat to public health despite the role of dogs as human companions. Additionally, a large number of stray dogs die from trauma, malnutrition, pyometra, uterine tumors, or other pathologies every year. An ovariohysterectomy (OVH) or spay is the surgical removal of the female reproductive organs. It includes the removal of ovaries, the uterine horns, and the body of the uterus (3,9). This surgical sterilization of bitch is the most frequently used method to prevent animal birth control (21) and to reduce the risk of mammary tumors (21,25), pyometra (8,21,24) and perianal fistulas (21). Though the OVH process has several disadvantages, complication as hemorrhage, wound healing, ovarian remnant syndrome, stump pyometra and ureteral injury (2,16,18), and surgical stress with behavioral change (7,11,20), broadly applied with different approaches (19,28).

A ventral midline approach (VMA) is used to conduct OVH in bitches. However, excessive mammary gland development is due to lactation or mammary gland hyperplasia (17). It is also reportedly linked to some post-operative complications, including excessive bleeding from the skin and subcutaneous tissue, wound inflammation or infection, leakage from mammary tissue (4), and evisceration of viscera (14). Various studies on surgical sterilizing techniques are accessible (5,10,14). The right flank approach (RFA) is the alternative to the ventral midline approach. However, relatively few studies have attempted to assess the comparative evaluation of different surgical and anesthetics parameters of VMA and RFA approaches and the post-operative problems accompanying OVH. The purpose of the study is to compare the different

parameters, viz. total duration of surgery, recovery time, length of incision, body temperature, heart rate, respiration rate, and SpO<sub>2</sub> at different time points in each approach.

## Materials and Methods

The study was conducted following the ethical guidelines of IASP and the WMA Declaration of Helsinki – Ethical protocol and approved by the University ethical committee of Agriculture and Forestry University (AFU), Nepal. Twenty (n = 20) healthy without any history of deformities, medications, non-pregnant mongrel bitches (Body condition score (BCS), Age and Weight of  $2.98 \pm 0.44$ ,  $2.58 \pm 1.03$  and  $15.69 \pm 5.41$ , respectively) were selected and isolated in kennel 24 hours before surgery for acclimatization. Physical examination, i.e. temperature, weight, heart rate (HR), pulse, respiratory rate (RR), mucous membrane and capillary refill time (CRT) and biochemical tests (complete blood count (CBC) and blood glucose level) were performed. The results were compared with standard reference ranges (26).

## Anaesthesia and medication

The bitches were fasted overnight, and standardized anesthesia protocols were used. The drugs used for the protocol are listed in Table 1. Each bitch was administered meloxicam as a preemptive analgesic half an hour before the induction, pre-medicated with atropine sulfate and xylazine-HCl followed by the induction with diazepam and ketamine-HCl IV, anesthesia was maintained with a maintenance dose of diazepam and ketamine-HCl combination. Fluid maintenance was done with RL. Depth of anesthesia was assessed subjectively from the jaw tone, the eyeball position, the pupil reflex,

**Table 1. Drugs used for 20 bitches**

Drugs	Indications	Dosage	Trade name	Route of administration
Meloxicam	Preemptive analgesia	0.02 mg/kg	Melonex, intas pharmaceutical Ltd	Subcutaneous (SC)
Atropine sulphate	Premedication, anticholinergic, parasympatholytic, reduces salivary secretion	0.02 mg/kg	Atropine sulphate injection, morvel laboratories Pvt. Ltd.	Subcutaneous (SC)
Xylazine-HCl	Premedication, sedation	2 mg/kg	Xyla, xylazine 2% injection, interchemie	Intramuscular (IM)
Diazepam	Muscle relaxant, general anesthesia	0.25 mg/kg (induction) 0.17 mg/kg (maintenance)	Lori, neon laboratories Ltd.	Intravenous (IV)
Ketamine-HCl	General anesthesia	2.5 mg/kg (induction) 3.33 mg/kg (maintenance)	KMINE, national healthcare	Intravenous (IV)
Lactated Ringer's solution	Fluid therapy	3 mL/kg/h	RL, varni corporation	Intravenous (IV)

tracheal reflex and the bitch's response to surgical manipulation reflexes. Prophylactic antibiotics ampicillin and cloxacillin (Megapen Inj, Aristo Pharmaceuticals) administration was done 15 min before induction. The operation was performed at room temperature.

## Surgery

The VMA group (n = 10) and RFA group (n = 10) were the two approaches of OVH used for this study. Bitches of both groups were surgically operated by standardized surgical protocol (1,12). A single surgeon with one assistant performed all surgeries. The fly repellent ointment (Himax, Indian Herbs) was topically applied over the surgical wound. Anesthesia recovery time and the post-operative temperature were recorded. The bitches were regularly monitored for the wound healing status and recorded the post-operative complication, if any.

## Parameters

Duration of surgery, recovery time, maintenance dose, clinical and physiological parameters recorded in clinical record sheet pre-operative after xylazine injection (T1), during operation (T2) and post-operative/recovery time (T3), 1 hour after induction (T4), 2 hours after induction (T5), 6 hours after induction (T6) and 24 hours after induction (T7). Also, intraoperative heart rate, respiration rate and SpO<sub>2</sub> were measured in different phases of OVH (phase zero: before incision, phase I: during incision to enter into the abdominal cavity, phase II: entry into the abdomen to access the right side ovary, phase III: ligation and severing of right side ovary to access to left side ovary, phase IV: ligation and severing of left side ovary to ligation and severing of the cervix, phase V: ligation of the cervix to the closing of the abdomen, phase VI: closing of skin, and phase VII: recovery). A sedation score (15) was used to study the sedation status from T1 to T7.

## Statistical evaluation

Examined data were recorded in Microsoft excel 10 and evaluated the mean ( $\mu$ ) and standard deviation (SD). Student t-test was conducted in SPSS program version 16 software, and statistical significance was checked (p-values < 0.05).

## Results

Preoperatively hemoglobin concentrations, total counts, differentiation of leukocytes, blood glucose and blood cortisol were within the reference intervals in all bitches. These results and the clinical examination confirmed that the bitches were healthy. The mean duration of surgery for VMA group

was significantly (p < 0.05) higher than RFA group. The mean recovery time for VMA group was significantly (p < 0.01) lower when compared to RFA group. During the study, a statistically insignificant difference in maintenance dose in VMA and RFA groups was observed. The mean length of surgical incision (cm) for VMA group was significantly (p < 0.01) longer when compared to RFA group. Both groups had no post-operative complications, and the wound condition was good. Surgical characteristics in terms of maintenance dose, duration of surgery, recovery time and length of incision were comparable for the VMA and RFA groups, as shown in Table 2. The mean rectal temperature at T1, T3 and T7 in VMA group was 101.04  $\pm$  0.33, 99.84  $\pm$  1.04, and 100.7  $\pm$  1.15, respectively. Similarly, in the RFA group, temperatures T1, T3 and T7 were 101.15  $\pm$  0.37, 100.43  $\pm$  0.76, and 100.99  $\pm$  0.53, respectively. A significant difference (p < 0.01) was recorded in recovery time (T3).

## Intra-operative heart rate (HR), respiratory rate (RR) and SpO<sub>2</sub> assessment

The heart rate, respiratory rate and SpO<sub>2</sub> concentration value (Mean  $\pm$  SD) in different time points undergoing VMA, and RFA surgery are shown in Table 3. The pre-operative values did not differ between the groups. The mean heart and respiratory rates were significantly increased during the severing of ovaries, ovarian ligaments and cervix compared with the T1 values only in the RFA group. No significant differences between and within groups for SpO<sub>2</sub> concentration were found.

## Sedation score

The level of sedation was found to be decreased with time intervals, and the bitches were completely recovered from

**Table 2.** Comparative physical and surgical characteristics values in mean  $\pm$  SD among VMA and RFA groups

Variables	VMA group	RFA group
BCS	2.9 $\pm$ 0.3	3.5 $\pm$ 0.5
Age (years)	2.4 $\pm$ 1.1	2.8 $\pm$ 1.0
Weight (kg)	15.4 $\pm$ 4.4	16.7 $\pm$ 6.3
Maintenance dose used (mL)	1.0 $\pm$ 1.2	1.6 $\pm$ 1.4
Total bitches that received the maintenance dose	7/10 (70%)	7/10 (70%)
Duration of surgery (minutes)	21.3 $\pm$ 5.5 <sup>a</sup>	16.5 $\pm$ 5.7
Recovery time (minutes)	9.3 $\pm$ 4.9 <sup>b</sup>	28.8 $\pm$ 13.1
Length of the surgical incision (cm)	3.5 $\pm$ 0.7 <sup>b</sup>	2.4 $\pm$ 0.8

<sup>a,b</sup>Significant differences in p < 0.05 and p < 0.01 respectively by t-test between groups.

**Table 3.** Heart rate, respiratory rate and SpO<sub>2</sub> concentration value (Mean ± SD) in different time points undergoing VMA and RFA OVH surgery

	Heart rate (beats per minute)		Respiratory rate (per minute)		SpO <sub>2</sub> concentration	
	VMA	RFA	VMA	RFA	VMA	RFA
Phase I	113.4 ± 8.1	104.4 ± 26.8	23.1 ± 6.7	23.3 ± 6.2	-	-
Phase II	116.1 ± 27.2	118.4 ± 27.9	21.4 ± 3.3	24.0 ± 6.0	91.8 ± 2.4	89.8 ± 6.7
Phase III	119.8 ± 32.4	134.4 ± 25.3 <sup>a</sup>	25.8 ± 6.5	27.5 ± 7.0 <sup>a</sup>	91.6 ± 2.5	91.2 ± 5.2
Phase IV	127.6 ± 24.0	152.5 ± 44.5 <sup>a</sup>	26.3 ± 7.9	27.8 ± 6.3 <sup>a</sup>	89.9 ± 4.4	90.4 ± 4.6
Phase V	127.2 ± 32.4	149.8 ± 32.4 <sup>a</sup>	27.2 ± 9.5	27.8 ± 5.8 <sup>a</sup>	92.5 ± 2.2	90.2 ± 5.3
Phase VI	119.9 ± 32.2	123.5 ± 23.1	26.1 ± 10.9	23.8 ± 3.6	90.4 ± 4.3	88.6 ± 6.9
Phase VII	110.1 ± 24.3	118.8 ± 21.3	26.2 ± 7.8	22.2 ± 4.1	-	-
24 hours	105.5 ± 12.7	117.5 ± 11.3	27.0 ± 5.3	25.5 ± 4.6	-	-

<sup>a</sup>Significant difference ( $p < 0.05$ ) by t-test within RFA groups with Phase I.

**Table 4.** Sedation scoring at different period of surgery

Groups	0 hr (T1)	1 hr (T4)	2 hrs (T5)	6 hrs (T6)	24 hrs (T7)
VMA	2.6 ± 0.5	1.2 ± 0.4 <sup>a</sup>	1.1 ± 0.5	0.6 ± 0.5	0
RFA	2.8 ± 0.4	1.7 ± 0.5 <sup>a</sup>	1.0 ± 0.0	0.5 ± 0.5	0

<sup>a</sup>Significant by one-way ANNOVA and t-test between the groups.

anesthesia within 24 hrs of surgery shown in Table 4. All the bitches were found significantly more sedated at 1 hr of surgery in the RFA than VMA ( $p = 0.05$ ).

## Discussion

Ventral midline and right flank approaches are the most common method of OVH. All the selected animals have no significant differences in detail (BCS, Age, and Weight). The duration of surgery for OVH in this study was less than those reported by Acharya et al. (1) that was  $49.4 \pm 0.59$  minutes (VMA) and  $26.2 \pm 0.76$  minutes (RFA), Hu et al. (13) was  $37.28 \pm 5.81$  (VMA) min, Sharda et al. (22) was  $44.25 \pm 3.50$  (VMA) and  $40.50 \pm 2.88$  (RFA). The variation in the duration of surgery might be due to the age, weight of the animals being operated and experience of the surgeons performing surgery. The recovery time of the RFA group was significantly higher than that of the VMA group. That may be due to the higher surgery duration of VMA. Furthermore, a higher recovery time in the RFA may be due to the sedative effects of the anesthetics were remained until recovery through short surgery duration. In this study, surgical incision length was lower than Sharda et al. (22) reported as  $8.17 \pm 0.65$  (VMA) and  $4.88 \pm 0.44$  (RFA). The reason for the shorter incision length in the flank approach was more accessible and practical to provide greater accessibility and location than the mid-

line approach (14). Postoperative/recovery time temperature was significantly lowered than pre-operative and 24 hrs after induction. This result was analogue to Shih et al. (23) and they reported pre-operative and post-operative temperature to be  $38 \pm 0.69$  and  $36 \pm 0.82$  degree Celsius respectively. Also, Davidson et al. (5) claimed that mild to moderate hypothermia, or hypotension was the primary intraoperative complication in both laparoscopic OVH and normal OVH, that the analogous to our study.

In the RFA group, the mean heart rate at the time of incision, severing of ovaries, cervix, and closing time was significantly higher than the pre-operative heart rate, which might be the cause of incision through the muscle layers in RFA. So, this indicates that RFA may have more pain or distress. Also, in VMA group heart rate increases non-significant in severing of second ovary and cervix. The elevation in heart rate in these animals might be due to some level of pain or distress; evoked by nociceptive afferent activity induced by tissue damage and manipulation, even in patients receiving adequate general anesthesia (6). Pre-operative and post-operative heart rate in our study was similar to the Shih et al. (23) ( $106 \pm 22$  and  $116 \pm 18$  beats per min respectively). In contrast to our study the baseline heart rate reported in a study conducted by Hu et al. (13) was higher i.e.  $124.1 \pm 10.9$  beats per minute and heart rate was significantly decreased with the baseline during the surgery ( $98.6 \pm 9.3$  beats per minute).

Significant ( $p < 0.05$ ) differences were found between the pre-operative respiratory rate with the time of severing of ovaries and cervix in the RFA group. Pre-operative and post-operative respiration rate in this study was higher than study Shih et al. (23) reported as  $15 \pm 7$  and  $13 \pm 8$  beats per minute, respectively. The baseline and post-operative respiration rate of present study is agreed with the Hu et al.

(13) that was  $23.3 \pm 1.9$  bpm and  $25.3 \pm 1.8$  bpm respectively but respiration rate after 24 hrs was  $27.6 \pm 3.2$  bpm that was slightly lower. Stretching and severing of ovarian ligament are painful procedures in both methods, significantly increased HR and RR were found during severing of suspensory ligament and cervix in the flank approach. The mean SpO<sub>2</sub> concentration had no significant ( $p > 0.05$ ) differences recorded in mean SpO<sub>2</sub> concentration between the groups and within groups. Warne et al. (27) reported that SpO<sub>2</sub> concentration was greater than 95% in cat undergoing VMA OVH, which was greater than this study. The pre-operative and post-operative SpO<sub>2</sub> concentration of the present study was less than that of Hu et al. (13) that was  $98.1 \pm 0.3\%$  and  $98.3 \pm 0.4\%$  respectively. Similar to the present study, Hu et al. (13) also had reported the non-significant changes in SpO<sub>2</sub> concentration. The level of sedation was found to be decreased with time interval, and the bitches were fully recovered from anesthesia at 24 hours of surgery. All the bitches were significantly higher sedated ( $p = 0.05$ ) at 1 hour of surgery in RFA than VMA ( $p = 0.05$ ). That may be due to more maintenance doses as compared to the midline.

In conclusion, compared to VMA, RFA had a statistical difference between minimal skin wounds and surgery duration and similarity with distress rate at the time of traction of the suspensory ligament and extended recovery time. Minimum duration and wound length in RFA infer less operating time and minimal tissue handling period. Thus, the study suggests that RFA is a better approach than VMA regarding duration and wound length. Further studies on other mammals, including bitches with molecular investigations of surgical stress, are imperative.

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### Conflicts of Interest

The authors have no conflicting interests.

### Ethical Declaration

The study was conducted following the ethical guidelines of IASP and the WMA Declaration of Helsinki – Ethical pro-

ocol and approved by the University Ethical Committee of Agriculture and Forestry University (AFU), Nepal.

## References

1. Acharya M, Sah MK, Singh DK, Singh S, Dhakal S. Comparative advantage of keyhole right flank laparotomy and ventral midline celiotomy for ovariohysterectomy in bitches. *Int J Appl Sci Biotechnol* 2016; 4: 198-202.
2. Adin CA. Complications of ovariohysterectomy and orchietomy in companion animals. *Vet Clin North Am Small Anim Pract* 2011; 41: 1023-1039, viii.
3. Asrat M, Melkamu S. Review on ovariohysterectomy: surgical approach, post-operative complications and their management in bitches. *Int J Adv Multidiscip Res* 2018; 5: 20-28.
4. Babu M, Krishnaswamy A, Nethra R, Narasimhamurthy. Comparison of two different laparotomy techniques for ovariohysterectomy and post-surgical complications in cats. *Int J Curr Microbiol App Sci* 2019; 8: 331-334.
5. Davidson EB, Moll HD, Payton ME. Comparison of laparoscopic ovariohysterectomy and ovariohysterectomy in dogs. *Vet Surg* 2004; 33: 62-69.
6. Dutta A, Maiti SK, Ajith P, Kumar N. Evaluation of different laparoscopic sterilization techniques in a canine birth control program. *Turk J Vet Anim Sci* 2010; 34: 393-402.
7. Fazio E, Medica P, Cravana C, Pupillo A, Ferlazzo A. Effects of ovariohysterectomy in dogs and cats on adrenocortical, haematological and behavioural parameters. *Acta Sci Vet* 2015; 43: 1339.
8. Fieni F, Topie E, Gogny A. Medical treatment for pyometra in dogs. *Reprod Domest Anim* 2014; 49 Suppl 2: 28-32.
9. Hancock R. Comparison of postoperative pain following ovariohysterectomy via harmonic scalpel-assisted laparoscopy versus traditional celiotomy in dogs. Blacksburg: Virginia Tech; 2005. [thesis].
10. Hancock RB, Lanz OI, Waldron DR, Duncan RB, Broadstone RV, Hendrix PK. Comparison of postoperative pain after ovariohysterectomy by harmonic scalpel-assisted laparoscopy compared with median celiotomy and ligation in dogs. *Vet Surg* 2005; 34: 273-282.
11. Hardie EM, Hansen BD, Carroll GS. Behavior after ovariohysterectomy in the dog: what's normal? *Appl Anim Behav Sci* 1997; 51: 111-128.
12. Howe LM. Surgical methods of contraception and sterilization. *Theriogenology* 2006; 66: 500-509.
13. Hu XY, Luan L, Guan W, Shi J, Zhao YB, Fan HG. Tolfenamic acid and meloxicam both provide an adequate degree of postoperative analgesia in dogs undergoing ovariohysterectomy. *Vet Med* 2017; 62: 333-341.
14. Kiani FA, Kachiwal AB, Shah MG, Nizamani ZA, Khand FM, Lochi

- GM, et al. Comparative study on midline and flank approaches for ovariohysterectomy in cats. *J Agric Food Tech* 2014; 4: 21-31.
15. Lascelles BD, Butterworth SJ, Waterman AE. Postoperative analgesic and sedative effects of carprofen and pethidine in dogs. *Vet Rec* 1994; 134: 187-191.
  16. Lee JM, Nam TC. Hyperestrogenic dermatitis after ovariohysterectomy in a dog. *J Vet Clin* 2001; 18: 448-451.
  17. McGrath H, Hardie RJ, Davis E. Lateral flank approach for ovariohysterectomy in small animals. *Compendium* 2004; 26: 922-930.
  18. Muraro L, White RS. Complications of ovariohysterectomy procedures performed in 1880 dogs. *Tierarztl Prax Ausg K Kleintiere Heimtiere* 2014; 42: 297-302.
  19. Mwangi WE, Mogoia EM, Mwangi JN, Mbuthia PG, Mbugua SW. A systematic review of analgesia practices in dogs undergoing ovariohysterectomy. *Vet World* 2018; 11: 1725-1735.
  20. Sakundech K, Chompoosan C, Tuchpramuk P, Boonsorn T, Aengwanich W. The influence of duration on pain stress, oxidative stress, and total antioxidant power status in female dogs undergoing ovariohysterectomy. *Vet World* 2020; 13: 160-164.
  21. Sanborn LJ. Long-term health risks and benefits associated with spay / neuter in dogs. New Brunswick: Rutgers University; 2007.
  22. Sharda R, Sahu D, Dewangan R, Panchkhande N, Sidar S, Yadav D. Conventional ventral midline and right flank approach for ovariohysterectomy in female dogs. *Indian J Anim Sci* 2022; 92: 565-569.
  23. Shih AC, Robertson S, Isaza N, Pablo L, Davies W. Comparison between analgesic effects of buprenorphine, carprofen, and buprenorphine with carprofen for canine ovariohysterectomy. *Vet Anaesth Analg* 2008; 35: 69-79.
  24. Smith FO. Canine pyometra. *Theriogenology* 2006; 66: 610-612.
  25. Sorenmo KU, Shofer FS, Goldschmidt MH. Effect of spaying and timing of spaying on survival of dogs with mammary carcinoma. *J Vet Intern Med* 2000; 14: 266-270.
  26. Stämpfli HR. MSD veterinary manual. Tetanus in horses. Rahway: Merck & Co., Inc. 2019.
  27. Warne LN, Beths T, Carter JE, Whittam T, Bauquier SH. Evaluation of the influence of atipamezole on the postoperative analgesic effect of buprenorphine in cats undergoing a surgical ovariohysterectomy. *Vet Anaesth Analg* 2016; 43: 424-428.
  28. Williamson JA, Johnson JT, Anderson S, Spangler D, Stonerook M, Dascanio JJ. A randomized trial comparing freely moving and zonal instruction of veterinary surgical skills using ovariohysterectomy models. *J Vet Med Educ* 2019; 46: 195-204.