TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(4): 393-402
© TÜBİTAK
doi:10.3906/vet-0908-24

Evaluation of different laparoscopic sterilization techniques in a canine birth control program

Avijit DUTTA, Swapan K. MAITI*, Parameswaran AJITH, Naveen KUMAR

Indian Veterinary Research Institute, Izatnagar 243 122, Bareilly (Uttar Pradesh) - INDIA

Received: 07.08.2009

Abstract: Three laparoscopic sterilization techniques under xylazine-ketamine anesthesia were evaluated at a 10 mmHg pressure gradient of CO_2 in 60 mongrel bitches, equally divided into 3 groups (A, B, and C). Laparoscopic bilateral oopherectomy was done in group A, laparoscopic ovariohysterectomy by endoclip and electrocautery in group B, and sterilization by electrocautery in group C. All of the animals recovered from anesthesia smoothly after the completion of laparoscopic surgery. Intraoperative observations revealed good visualization of the reproductive organs at 10 mmHg of pressure in the Trendelenburg position. Comparison among the various laparoscopic techniques revealed a more effective hemostasis with titanium endoclip and electrocautery in the animals of groups A and B. No immediate postlaparoscopic complications were observed in any animal. Hemato-biochemical and physiological parameters did not reveal any significant changes in these animals during the postoperative period. In all of the groups, effective removal of ovaries was correlated with a postoperative decrease of plasma estrogen and progesterone level. Laparoscopic ovariohysterectomy by endoclip and electrocautery was found to be better than the other 2 techniques.

Key words: Capnoperitoneum, dog, laparoscopic oopherectomy, laparoscopic ovariohysterectomy, sterilization

Introduction

Among the various modern surgical methods, laparoscopy is now held as one of the most potent and promising aids for both its diagnostic (laparoscopy) and therapeutic (laparoscopic surgery) use. A laparoscope is introduced into the body cavity to capture the various images of the internal organs by a specialized camera, and then it transmits the images to the television screen or monitor. Thus, it explores a new modality to intervene between the intraabdominal organs in an easier and less stressful way for therapeutic necessities.

It has been established that laparoscopic surgery provides some distinct advantages over conventional laparotomy. It involves minimal invasiveness (keyhole surgery) with maximum visibility, shorter surgical time, decreased postoperative discomfort and pain, less incidence of infection, uncomplicated healing with minimal scarring, and minimal surgical morbidity (1,2). Intraoperative hemostasis can be successfully achieved by ligatures, stapling, and electrocautery (3). Laparoscopy is now emerging as a diagnostic and therapeutic tool in the veterinary field.

* E-mail: maiti@ivri.up.nic.in

In addition, laparoscopic surgery provides a wide field of extensive application, particularly in the sterilization of different species of animals. Demographically dense urban and industrial areas require effective animal birth control programs that can overcome the problems of hospitalization, postoperative complications, and reduction in the overall cost of operation. In a large scale animal birth control program, the conventional methods of sterilization require a long period between capture of dogs and their release, due to the time taken for the surgical wounds to heal. In this aspect, keyhole surgery (laparoscopic surgery) can revolutionize the entire program, as it needs only a very small surgical wound, which usually needs no postoperative care or regular dressings. The available literature reports a few laparoscopic sterilization techniques in bitches (4,5), but no single technique has been reported as a standard routine sterilization program, keeping in mind the minimum operative time and pain and stress to the patient. Therefore, the present study was undertaken to evaluate 3 different laparoscopic sterilization techniques in a canine birth control program.

Materials and methods

The study was conducted on 60 clinically healthy adult female dogs with body weights of 15-20 kg (17.20 ± 0.62) and ages of 16-28 months (20.68 ± 1.28). The animals were randomly divided into 3 equal groups (A, B, and C) consisting of 20 animals each. In group A, laparoscopic bilateral oopherectomy was performed. In group B, laparoscopic ovariohysterectomy by endoclip and electrocautery was performed, and in group C, laparoscopic ovariohysterectomy by electrocautery only was performed. After administration of anesthesia, the animals were placed in dorsal recumbency and then in the Trendelenburg position for laparoscopic oopherectomy or ovariohysterectomy.

A small 0.5 cm skin incision was made at the level of the umbilicus and a Veres needle was inserted. Insufflation of the abdominal cavity was achieved with carbon dioxide gas at the rate of 2 L/min with a pressure gradient of 10 mmHg. A 6 mm safety trocar and cannula unit was inserted into the abdominal cavity. A rigid-type telescope (30 degrees, 5 mm in diameter, Frontline Co., Germany) connected to a light source (40 W, halogen lamp) and a digital camera was then introduced through the cannula. The intraperitoneal organs were visualized. The urinary bladder was identified first by its characteristic tortuous structures of blood vessels. The uterus and ovarian structure were thoroughly visualized with their characteristic ivory-colored, cord-like structure (Figures 1 and 2). Two paramedian ports of 10 mm were created under the guidance of the telescope, distal to the laparoscope insertion site and 4-6 cm bilaterally from the ventral midline for insertion of the operative instruments.

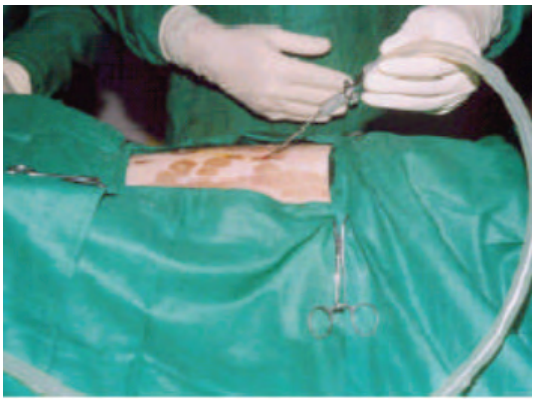

Figure 1. Insertion of Veres needle into the abdomen for pneumoperitoneum.

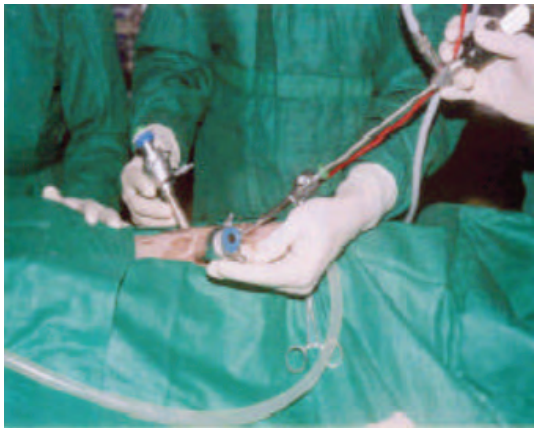

Figure 2. Arrangement of 3 trocars outside the abdomen.

In animals in group A, fenestrated grasping forceps were placed through the right paramedian port, and the right ovary was pulled caudally to visualize the right ovarian ligament and vessels properly. The clip applicator, preloaded with a titanium endoclip, was inserted through the left paramedian port and then the first endoclip was applied around the cranial ovarian ligament, including the vessels. A second endoclip was applied on the uterine horn 0.5 cm away from the ovarian bursa, and then the clip applicator was removed. Laparoscopic monopolar scissors were then inserted through the left paramedian port to resect and cauterize the uterine horn, broad ligament, and mesovarium structure; it was attached with an electrocautery unit. A 60 W monopolar current was used for cutting and cauterization. The total ovarian mass was dislodged and removed through a 10 mm port. The opposite ovary was removed in the same manner (Figures 3-5).

In animals in group B, the right ovary was grasped by fenestrated forceps inserted through the right paramedian port. A clip applicator was inserted through the left side of the paramedian port; the endoclip was applied around the cranial ovarian ligament and vessels, and then it was removed. Laparoscopic monopolar scissors that were attached with an electrocautery unit were then inserted through the left side port to resect and cauterize the cranial ovarian ligament, suspensory ligament, ovarian pedicle, and broad ligament of the uterus up to the level of the uterine body. A 60 W monopolar current was used for cutting and cauterization. The uterine artery, vein, and uterine body, proximal to the cervix, were also clipped, transected, and coagulated in the same manner. The procedure was repeated for the left ovary up to the level of the cranial suspensory ligament. Finally, the completely resected utero-ovarian structure was removed through a 10 mm port (Figures 6-8).

The technique employed in animals in group C was the same as that described in group B, except that the endoclip application and hemostasis was achieved in this group only by cutting and cauterizing the utero-ovarian structure at the same level (Figures 9-12).

All of the animals were assiduously inspected at the resected sites for hemorrhage after completion of the procedure. After thorough examination, the telescope and endocamera were taken out. Intraabdominal CO_2 gas was allowed to escape through the cannula. The incisions were sutured with 1 or 2 simple interrupted sutures. Antiseptic dressing was applied regularly for 3 days postsurgery. The animals were evaluated on the following observations.

Intraoperative and postoperative observations:

The laparoscopic surgical techniques were evaluated based on the flow rate and total utilization of carbon dioxide for each operation, the instruments required, organ manipulation and maneuverability, intraoperative complications, and surgical operating

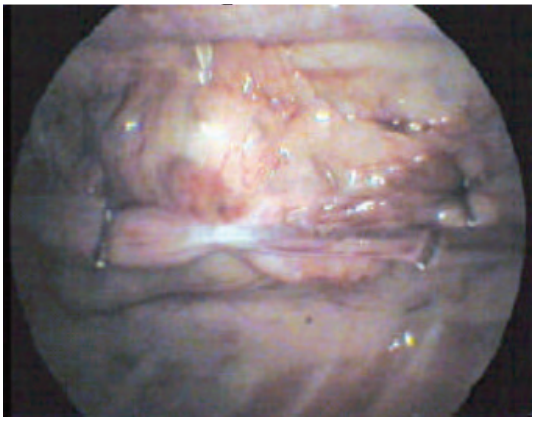

Figure 3. Application of endoclip on both sides of ovary in the animals of group A.

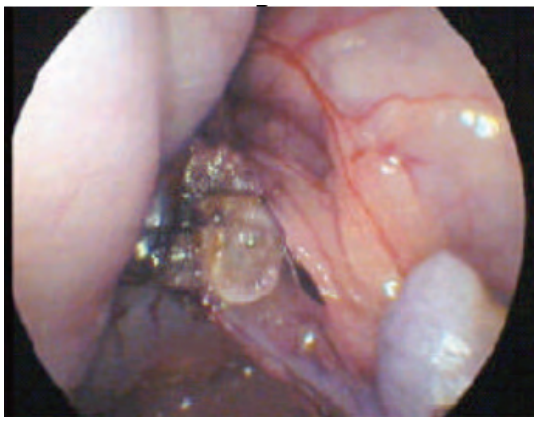

Figure 4. Complete resection of ovarian attachment in the animals of group A.

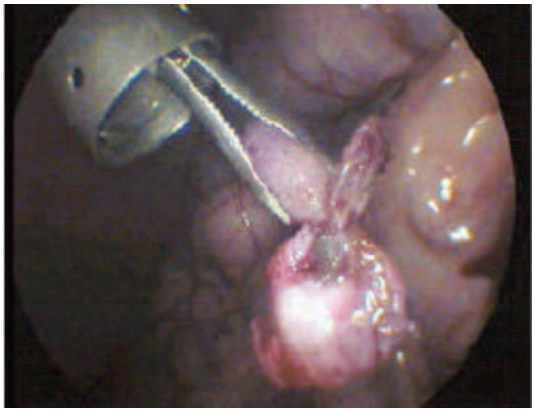

Figure 5. Removal of resected ovaries outside of the abdomen in group A.

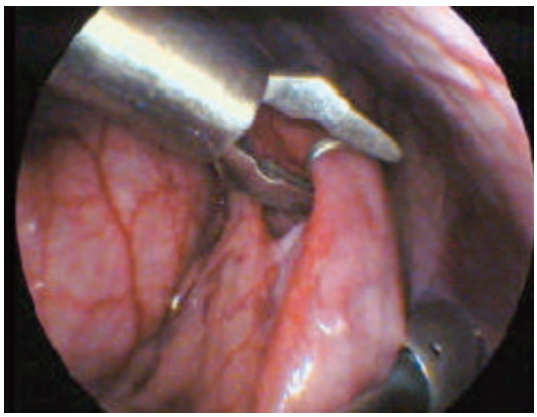

Figure 6. Clipping of uterine body cranial to cervix in the animals of group B.

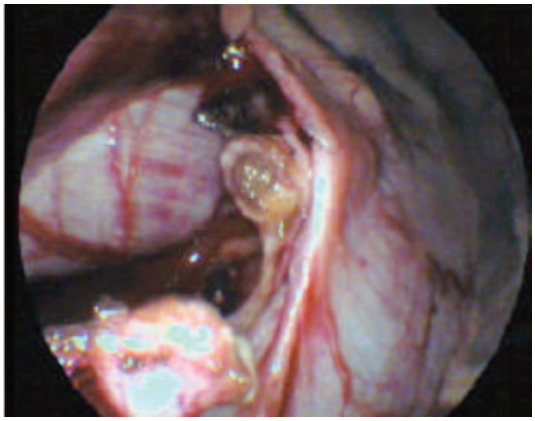

Figure 7. Complete resection of uterine body in the animals of group B.

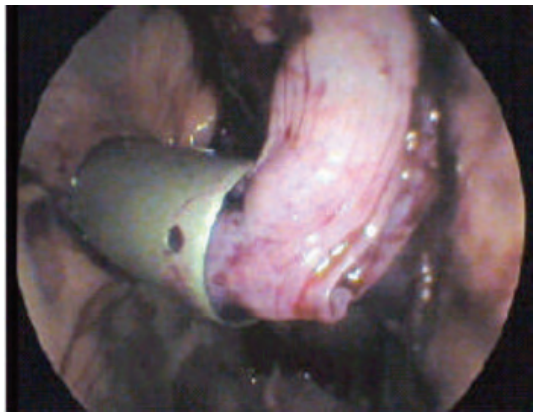

Figure 8. Removal of resected utero-ovarian body outside of the abdomen in group B.

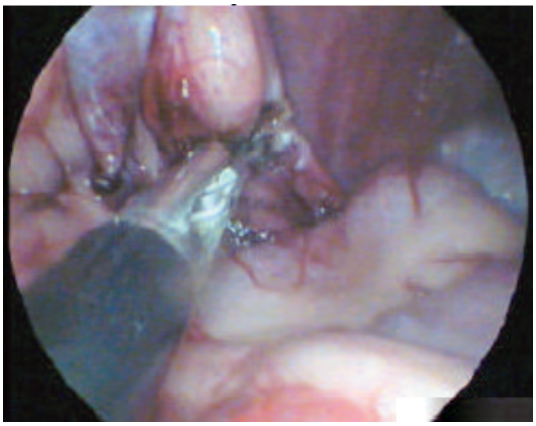

Figure 9. Cauterization and resection of cranial ovarian attachment in the animals of group C.

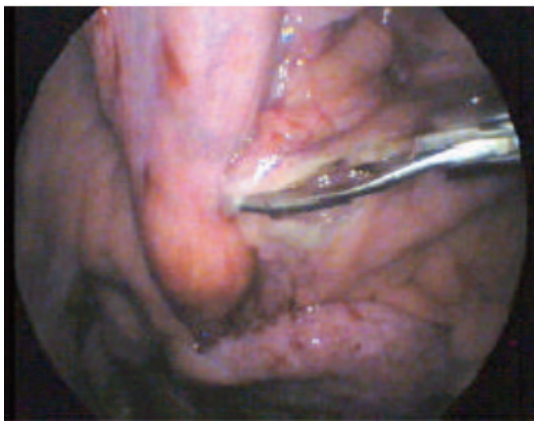

Figure 10. Cauterization and separation of mesometrium in the animals of group C.

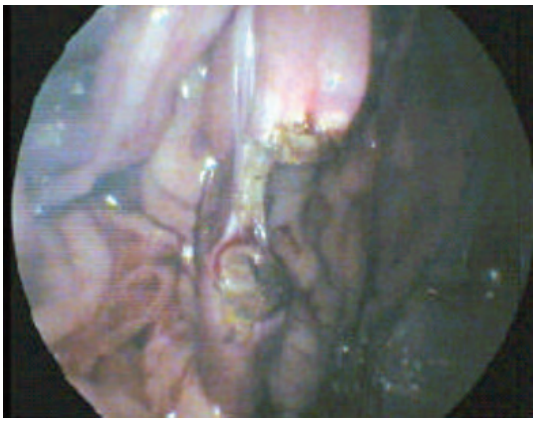

Figure 11. Complete separation of uterine body in the animals of group C.

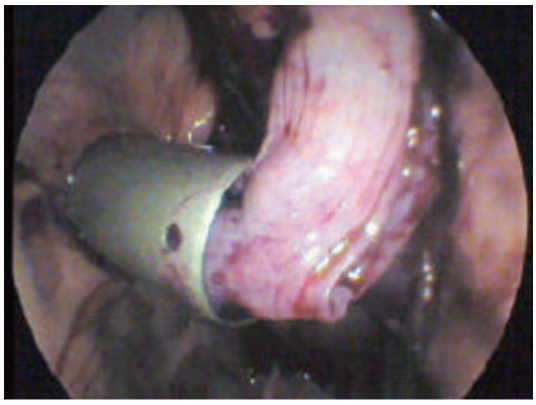

Figure 12. Removal of resected utero-ovarian body outside of the abdomen in group C.

time, which was defined as the time from the beginning of the first incision up to the last skin suture. General behavior, including discomfort and uneasiness, changes in feeding habits, defecation and urination, and licking of the suture site, was observed. Each animal in the 3 groups was carefully monitored for complications like emphysema, port site herniation, bacterial peritonitis, ascites, and stitch abscess.

Clinical observations:

The respiratory rate (breaths/min), heart rate (beats/min), and rectal temperature (°F) were recorded before the start of the operation and on days 1, 3, 5, and 7 after surgery.

Biochemical observations:

Heparinized blood was collected before the start of the operation and on days 1, 3, 5, and 7 after surgery for estimation of alkaline phosphatase and acid phosphatase.

Hormonal estimation:

The plasma samples were used to estimate the cortisol, insulin, thyroxine (T_4), estrogen, and progesterone hormone with a radioimmunoassay (RIA) kit.

The data were subjected to 2-way analysis of variance (ANOVA) and the paired t-test, as per standard statistical methods.

Results

Intraoperative and postoperative observations

The surgical phase of anesthesia in all of the animals of the different groups was achieved by administering xylazine and ketamine in combination. In group A, no additional anesthesia was required in any animal to complete the surgical procedure. In contrast, a second dose of ketamine and diazepam combination was needed in all of the animals of groups B and C to prolong the anesthetic stage. The postsurgical recovery from anesthesia in all of the groups was smooth and uneventful. Establishment of capnoperitoneum (CP) in each animal was easy and safe. The 10 mmHg pressure gradient was adequate to perform laparoscopic surgery in the different groups. The CO_2 flow rate of 2 L/min was also sufficient to maintain intraabdominal pressure during surgery. Utilization of CO_2 gas was significantly higher ($P < 0.01$) in groups B and C than it was in group A.

Mean ± SE value of total CO_2 utilized (L) in animals of different groups:

Group	A	B	C
Total CO_2 utilized	13.74 ± 2.47	41.08 ± 4.16	43.96 ± 2.89

For laparoscopic sterilization in these 3 groups, 3 ports were sufficient to conduct the sterilization procedure. A monopolar coagulation current of 60 W was effective for electrocautery as well as coagulation of the line of cutting of the utero-ovarian structures. There was good exposure and visibility of the utero-ovarian structures and uterine blood vessels and broad ligament. Lifting the uterus or ovary by fenestrated grasping forceps facilitated accuracy in cutting and cauterization. Different types of instruments were needed during laparoscopic oopherectomy in group A and laparoscopic ovariohysterectomy in groups B and C, and it differed according to the aim of surgery. In groups A and B, fenestrated grasping and dissecting forceps, scissors, and clip applicators were required. In group C, only the fenestrated forceps and scissors were required. In group A, 3 animals revealed evidence of minor hemorrhage from a transected mesovarium even after the clipping and cauterization. Minute hemorrhaging was also found at the resection site near the uterine stump in 3 animals of group B and 12 animals of group C. Repeated applications of coagulation current were needed to achieve complete hemostasis. All of the animals were closely monitored for effective hemostasis before the final withdrawal of the telescope. Another minute complication encountered in this study was accidental touch and thereby minor damage of the organ during electrocauterization in 1 animal of group A and 2 animals of group C. However, this minor trauma did not affect the animal during or after surgery. Surgical operating time of group A was significantly ($P < 0.01$) lower than that of groups B and C. Groups B and C had more or less similar operating times.

Mean ± SE of surgical operating time (min) in 3 groups:

Group	A	B	C
Surgical operating time	25.00 ± 3.46	50.83 ± 5.30	47.17 ± 4.13

Normal appetite returned within 8-10 h after operation in all animals. Oliguria was observed in all animals for an initial 12 h after surgery. However, defecation was normal throughout the observation period. All animals appeared quite alert and responsive throughout the postoperative period. No postoperative complications like emphysema, portal herniation, peritonitis, ascites, or stitch abscess were recorded in any animals of the 3 groups.

Clinical observations:

Respiration rate (breaths/min) in all animals remained within the normal limit throughout the observation period. A statistically significant decrease ($P < 0.05$) in respiration rate immediately after the operation was observed in all 3 groups. No significant difference ($P > 0.05$) was observed in respiration rate when it was compared among the 3 groups. Heart rate (beats/min) decreased nonsignificantly ($P > 0.05$) immediately after the operation in all 3 groups. Preoperative as well as postoperative mean rectal temperatures (°F) recorded in all 3 groups remained within the normal limits throughout the observation period.

Biochemical observations:

A nonsignificant increase ($P > 0.05$) in ALP and ACP levels was observed on the third postoperative day in all 3 groups. Later, it decreased and returned to base values on day 7. However, no significant difference among the groups was observed at different time intervals.

Hormonal estimation:

The preoperative plasma cortisol level did not show any significant change among the groups. A nonsignificant increase ($P > 0.05$) in cortisol levels was observed immediately after the operation in all 3 groups. In subsequent intervals, the cortisol levels decreased and returned to base values on day 7. However, no significant difference among the groups was observed at different time intervals. The preoperative plasma insulin levels were within normal limits in all animals of the 3 groups. No significant ($P > 0.05$) difference in insulin levels was observed during the postoperative days. Preoperative and postoperative thyroxine (T_4) values among the 3 groups did not show any significant difference ($P > 0.05$). However, a nonsignificant ($P > 0.05$) decrease in

thyroxine levels was observed in groups B and C on the first postoperative day. In subsequent intervals, the thyroxine levels increased and returned to base values on day 7 postoperatively. Preoperative plasma estrogen and progesterone values of the 3 groups did not show any significant difference. However, in all 3 groups, a significant ($P < 0.01$) decrease in plasma estrogen and progesterone level was observed immediately after the operation, and thereafter the values showed a gradual decrease ($P > 0.05$) up to the seventh postoperative day.

Discussion

Laparoscopy is an endoscopic procedure that bridges the gap between clinical evaluation and surgical exploration. The laparoscopic surgical techniques in both human and veterinary medicine have grown tremendously. Laparoscopy requires a minor surgical intervention and it provides the only available practical means of making repeated direct observation of abdominal viscera (2). The advantages of surgical laparoscopy over the conventional open surgical exploratory laparotomy include faster patient recovery because of smaller surgical sites, decreased hospitalization, improved cosmesis, improved visualization of abdominal organs, lower postoperative morbidity with lower infection rates, and less postoperative pain and stress (6). Surgeons are continually looking for more progressive and less stressful surgical methods for sterilization in dogs, particularly for canine birth control programs in urban and industrial areas.

In this study, 3 laparoscopic sterilization techniques in female dogs were compared and evaluated. All of the laparoscopic surgical procedures were conducted under xylazine and ketamine general anesthesia. Induction as well as recovery from general anesthesia was smooth and uneventful in all of the animals, as was also reported by Wildt et al. (1) in using this combination of anesthesia for direct observation of internal organs and for the laparoscopic sterilization of male dogs.

During laparoscopic sterilization of the animals of groups A, B, and C, CO_2 pneumoperitoneum or capnoperitoneum (CP) was established at 10 mmHg pressure gradients intraabdominally. The initial flow rate of carbon dioxide at 2 L/min was sufficient to achieve CP. This pressure and flow rate provided adequate inflation and excellent working space, as was also observed by Dharmaceelan et al. (7). The utilizations of CO_2 in groups B and C were significantly higher than in group A. The minimal utilization of CO_2 in group A could be due to the reduced time taken to perform bilateral oopherectomy, as it is a comparatively simple and quicker method. Three ports were adequate to conduct the laparoscopic bilateral oopherectomy in group A, as well as the laparoscopic ovariohysterectomy in groups B and C. Two 10 mm ports were created at the left and right paramedian site distally to the telescope insertion site and 4-6 cm laterally at the inguinal regions. The remaining port (5 mm) was at the umbilical site for insertion of the telescope, as was also reported by Wildt and Lawler (8). The urinary bladder was visualized first with the introduction of the telescope into the abdominal cavity; it was identified by its characteristic tortuous structures of blood vessels. Most clinicians have reported the use of a 5-mm telescope during ovariohysterectomy in dogs (7,9), laparoscopic occlusion of the ductus deferens in male dogs and cats (8), and laparoscopic vasectomy in male dogs (10). Proper fasting prior to surgery emptied the intestine and urinary bladder and thereby facilitated proper visualization of the utero-ovarian structures, ovarian blood vessels, and broad ligaments (9,11). Electrocautery with a 60 W monopolar current revealed a good hemostatic measure in groups A and B, where bilateral oopherectomy and ovariohysterectomy was applied, respectively. In both groups, 3 animals were found to have minor hemorrhage from the uterine or ovarian stump immediately after resectioning. Complete hemostasis was achieved in these animals by a second application of coagulation current. This study concurred with the findings of Uson et al. (12) and Brun et al. (13). In group C, where ovariohysterectomy was done only by electrocautery of the utero-ovarian site without endoclipping, hemostasis was less satisfactory in comparison with groups A and B. Among the 20 animals in this group, 12 animals had repeated electrocautery for effective hemostasis. Van Goethem et al. (14) reported a higher percentage of postresection mesovarium arterial bleeding with monopolar cautery, whereas Rodgerson et al. (15) also observed that monopolar electrocautery alone was

sufficient for effective hemostasis in equine mesovarium cauterization. On the other hand, Dharmaceelan et al. (7) reported that monopolar cautery along with endoclipping was better than cautery alone. However, before final withdrawal of the telescope, all of the animals of group C were closely observed at the resected site and no further complications were observed.

The complication observed in the study was a mild thermal injury during cauterization. It happened with a part of the large intestine in 1 animal of group A and with the urinary bladders in animals of group C. However, these traumas were uneventful on the part of the animals. Malm et al. (16) reported splenic lesions in 3 out of 30 animals during ovariohysterectomy. Minor complications like splenic laceration and vaginal discharge were observed by Davidson et al. (5) in 9 out of 16 laparoscopic ovariohysterectomy patients. Thus, this study appeared to have fewer laparoscopic complications in comparison with other studies.

Mean surgical operating time was significantly lower in group A as compared with groups B and C. The higher operative times recorded in groups B and C were due to the time taken for the cauterization and resectioning of the greater utero-ovarian tissues. In the animals of group B, application of the endoclip also contributed to lengthening the surgical period, whereas, in group C, additional time was needed for proper monitoring of the resected site to combat the problem of hemorrhage. The operative time for bilateral oopherectomy in group A was lower in comparison with that reported by Dharmaceelan et al. (7). The operative time required for laparoscopic ovariohysterectomy in this study was a little more than that reported by Davidson et al. (5), but less than that reported by Hancock et al. (17).

All of the animals returned to their normal feeding habits within 8-10 h of surgery. Urination and defecation were normal up to the seventh postoperative day. The animals appeared quite alert and responsive. No postoperative emphysema, port site herniation, or wound dehiscence was observed in any animal. All of these observations reflected the early recovery of the animals after laparoscopic sterilization operation.

In clinical observations, no significant differences in physiological parameters such as heart rate, respiration rate, or rectal temperature at different time intervals were observed. Heart rate, respiration rate, and rectal temperature did not change significantly after surgery, and so these variables cannot be considered useful in the recognition of postoperative pain (18).

The plasma ALP levels in all animals were within the normal limits, but a nonsignificant increase in this enzyme was observed on the third postoperative day in all 3 surgical groups. The elevation in plasma ALP after laparoscopic surgery in these animals may be attributed to tissue injury as a result of ischemia/reperfusion-induced oxidative stress in the liver and kidneys following CP (19). Acid phosphatase (ACP) activity was reported to be derived from lysosomal compartments of cells, predominantly in the bone and to some extent in other cells like platelets, erythrocyte, and spleen (20). A nonsignificant increase in plasma ACP was observed on the first and third postoperative days, but there was no significant difference among the groups. Changes in serum ACP levels could not be correlated with the type of tissue injury that occurred in different groups because it is a weak marker of soft tissue injury.

Surgically induced stress responses are evoked by nociceptive afferent activity induced by tissue damage and manipulation, even in patients that are receiving adequate general anesthesia (21). In the present study, a nonsignificant elevation in plasma cortisol that was observed on the first postoperative day in all groups was probably due to the effect of pneumoperitoneum rather than operative trauma (22). A significant increase in plasma cortisol levels in response to surgical stress in dogs and cats after ovariohysterectomy was reported by Fox et al. (23) and Smith et al. (18), respectively. Marcovich et al. (22) reported a higher cortisol level at 4 h, whereas the peak cortisol level after laparoscopy in dogs was observed at 2 h by Hancock et al. (17).

Hormonal responses to injury and stress have been reflected through the change in plasma insulin level (24). However, in this study, no significant difference in plasma insulin levels was observed in any of the 3 groups at different time intervals. Benson et al. (25) reported higher insulin levels following conventional ovariohysterectomy.

Plasma thyroxine (T_4) is a good adjunct to evaluate the surgical stress (26). The plasma thyroxine levels were within the normal limits in group A, but a nonsignificant decrease in this hormone was observed up to the fifth postoperative day in the animals of groups B and C, possibly due to the effect of prolong anesthesia and more surgical stress, as reported by Sari and Sevinc (27) after performing laparoscopic surgery.

Estrogen and progesterone are 2 female reproductive hormones often used to evaluate the reproductive status of an animal. The ovaries are the major source of these hormones (28). Due to the source specificity, their estimation has been indicated to assess the effects of surgical techniques following ovariohysterectomy (29). In all 3 groups, a significant decrease ($P < 0.01$) in plasma estrogen and progesterone was observed immediately after the operation, up to the seventh postoperative day, which was attributed to the effective removal of its major source, i.e. ovarian tissue, during the different sterilization operations in the 3 groups.

The results of this study indicated that laparoscopic ovariohysterectomy by endoclipping and electrocautery in dogs provided optimum hemostasis and effective removal of utero-ovarian structures.

References

1. Wildt, D.E., Kinney, G.M., Seager, S.W.J.: Laparoscopy for direct observation of internal organs of domestic cats and dogs. Am. J. Vet. Res., 1977; 38: 1429-1432.
2. Maiti, S.K., Dutta, A., Ajith, P., Kumar, N., Sharma, A.K., Singh, G.R.: Laparoscopy for direct examination of internal organs in small animals. J. Appl. Anim. Res., 2008; 33: 201-204.
3. Willimzik, H.F., Weber, K.O.: Electrocoagulation sealing of ovarian blood vessels during ovariectomy of cats. Kleintier-Praxis, 1992; 37: 539-540. (article in German with an abstract in English)
4. Minami, S., Okamoto, Y., Eguchi, H., Kato, K.: Successful laparoscopy assisted ovariohysterectomy in two dogs with pyometra. J. Vet. Med. Sci., 1997; 59: 845-847.
5. Davidson, E.B., Moll, H.D., Payton, M.E.: Comparison of laparoscopic ovariohysterectomy and ovariohysterectomy in dogs. Vet. Surg., 2004; 33: 62-69.
6. Rothuizen, J.: Laparoscopy in small animal medicine. Vet. Q., 1985; 7: 225-228.
7. Dharmaceelan, S., Ganesh, T.N., David, W.P.A.: Laparoscopic sterilization in bitches: comparison of three techniques. Indian J. Vet. Surg., 2000; 21: 65-68.
8. Wildt, D.E., Lawler, D.F.: Laparoscopic sterilization of the bitch and queen by uterine horn occlusion. Am. J. Vet. Res., 1985; 46: 864-869.
9. Wildt, D.E., Seager, S.W.J., Bridges, C.H.: Sterilization of the male dog and cat by laparoscopic occlusion of the ductus deferens. Am. J. Vet. Res., 1981; 42: 1888-1897.
10. Silva, L.D., Onclin, K., Donnay, I., Verstegen, J.P.: Laparoscopic vasectomy in the male dog. J. Reprod. Fertil. Suppl., 1993; 47: 399-401.
11. Thiele, S., Kelch, G., Gerlack, K.: Castration of the bitch by means of laparoscopical ovariectomy. Kleintier-Praxis, 1993; 38: 463-466.
12. Uson, J., Tejedo Vives, M.A., Ezguerra, L.J., Uson, J.M.: Therapeutique laparoscopique: l'ovariectomie et la legature des cornes uterines chez la chienne. Rec. Med. Vet., 1992; 168: 237-241.
13. Brun, M.V., Silva Filho, A.P.F., Beck, C.A.C., Mariano, M.B., Mello, J.R.B.: Laparoscopic ovariohysterectomy in canine. Braz. J. Vet. Res. Anim. Sci., 2000; 37: 480-485.
14. Van Goethem, B.E., Rosenveldt, K.W., Kirpensteijn, J.: Monopolar versus bipolar electrocoagulation in canine laparoscopic ovariectomy: a nonrandomized, prospective, clinical trial. Vet. Surg., 2003; 32: 464-470.
15. Rodgerson, D.H., Belknap, J.K., Wilson, D.A.: Laparoscopic ovariectomy using sequential electrocoagulation and sharp transection of the equine mesovarium. Vet. Surg., 2001; 30: 572-579.
16. Malm, C., Savissa-Rocha, P.R., Gheller, V.A., Oliveira, H.P., Lamounier, A.R., Foltyneck, V.: Ovariohysterectomy: experimental and comparative study between laparoscopic and conventional approaches. Intra-operative phase. Arq. Bras. Med. Vet. Zootec., 2004; 56: 457-466.
17. Hancock, R.B., Lanz, O.I., Waldron, D.R., Duncan, R.B., Broadstone, R.V., Hendrix, P.K.: 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.
18. Smith, J.D., Allen, S.W., Quandt, J.E.: Changes in cortisol concentration in response to stress and postoperative pain in client-owned cats and correlation with objective clinical variables. Am. J. Vet. Res., 1999; 60: 432-436.
19. Tan, M., Xu, F.F., Peng, J.S., Li, D.M., Chen, L.H., Lv, B.J., Zhao, Z.X., Huang, C., Zheng, C.X.: Changes in the level of serum liver enzymes after laparoscopic surgery. World J. Gastroenterol., 2003; 9: 364-367.

20. Rosol, J.T., Capen, C.C.: Calcium regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L., Eds. Clinical Biochemistry of Domestic Animals. 5th edn., Academic Press, London, 1997; 619-702.

21. Hume, D.M., Egdahl, R.H.: The importance of the brain in the endocrine response to injury. Ann. Surg., 1959; 150: 697-712.

22. Marcovich, R., Williams, A.L., Seifman, B.D., Wolf, J.S. Jr: A canine model to assess the biochemical stress response to laparoscopic and open surgery. J. Endourol., 2001; 15: 1005-1008.

23. Fox, S.M., Mellor, D.J., Firth, E.C., Hodge, H. Lawoko, C.R.O.: Changes in plasma cortisol concentrations before, during and after analgesia, anaesthesia and anaesthesia plus ovariohysterectomy in bitches. Res. Vet. Sci., 1994; 57: 110-118.

24. Newsome, H.H. Jr: Neuroendocrine system. In: Bruke, J.F. Ed. Surgical Physiology. W. B. Saunders Co., Philadelphia, 1983; 208-225.

25. Benson, G.J., Grubb, T.L., Neff-Davis, C., Olson, W.A., Thurmon, J.C., Lindner, D.L., Tranquilli, W.J., Vanio, O.: Perioperative stress response in the dog: effect of pre-emptive administration of medetomidine. Vet. Surg., 2000; 29: 85-91.

26. Kirby, R., Clark, F., Johnston, I.D.A.: The effect of surgical operation of moderate severity on thyroid function. Clin. Endocrinol., 1973; 2: 89-99.

27. Sari, R., Sevinc, A.: The effects of laparoscopic cholecystectomy operation on C-reactive protein, hormones, and cytokines. J. Endocrinol. Invest, 2004; 27: 106-110.

28. Pineda, M.H.: Female reproductive system. In: Pineda, M.H., Dooley, M.P., Eds. McDonald's Veterinary Endocrinology and Reproduction. Iowa State Press, Iowa, 2003; 283-340.

29. Edqvist, L.E., Forsberg, M.: Clinical reproductive endocrinology. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L., Eds. Clinical Biochemistry of Domestic Animals. Academic Press, London, 1997; 589-617.