TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2009; 33(5): 427-436
© TÜBİTAK
doi:10.3906/vet-0805-30

Laparoscopic sterilization vs. open method sterilization in dogs: a comparison of two techniques

Anburaja MAHALINGAM, Naveen KUMAR*, Swapan K. MAITI, Ashok K. SHARMA,
Umesh DIMRI, Meena KATARIA

Division of Surgery, Indian Veterinary Research Institute, Izatnagar-243122 - INDIA

Received: 29.05.2008

Abstract: Ten clinically healthy, adult male dogs randomly equally divided into 2 groups (I and II) were subjected to laparoscopic sterilization and open method castration under xylazine-ketamine anesthesia. In group I laparoscopic vasectomy by cauterization and cutting of the vas deferens was performed and in group II conventional open castration by the pre-scrotal approach was done. Insufflation of the abdominal cavity was achieved with CO_2 (2 L/min) at 10 mmHg pressure gradient. Two ports were needed to carry out the operation. Clinical observations revealed no significant changes. Differential leukocyte count (DLC) revealed significant neutrophilia and comparative lymphopenia on the 3rd postoperative day in both the groups. A significant increase ($P < 0.05$) in plasma alkaline and acid phosphatase level was observed in both the groups on day 3 postoperatively. Indices of oxidative stress viz. lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), reduced glutathione activity and acute phase protein, and ceruloplasmin level in plasma did not reveal any major significant changes. Plasma cortisol level did not show any significant change in group I whereas in group II the level increased significantly ($P < 0.01$) after the operation. No significant change in testosterone level was observed in group I whereas in group II a significant decrease ($P < 0.01$) was observed immediately after the operation. On the basis of the parameters studied it can be concluded that early healing and better cosmoses were achieved by laparoscopic sterilization (vasectomy) in male dogs as compared to the conventional open method of castration and the technique can be successfully applied for mass sterilization programs.

Key words: Male dogs, laparoscopic vasectomy, sterilization, open method castration

Introduction

Laparoscopic surgery provides a wide field of extensive applications particularly in surgical sterilization of different animal species. The sterilization of both males and females is an effective measure for controlling the stray dog problem. Castration of male dogs by the conventional open method has many disadvantages and postoperative complications such as hemorrhage, wound dehiscence, infections, maggot infestations, and scrotal swellings. 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. It also avoids postoperative complications such as wound dehiscence and herniation, and

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

reduces the surgical stress to animal as well as the recurring cost of each surgery. Laparoscopic surgery requires minimal invasiveness (keyhole surgery) with maximum visibility, shorter surgical time, decreased postoperative discomfort and pain, less incidences of infection, uncomplicated healing with minimal scarring, and minimal surgical morbidity (1). A review of the literature reveals the paucity of reports regarding laparoscopic sterilization in male animals. Therefore, the present study was undertaken to standardize the laparoscopic sterilization techniques in male dogs and to compare laparoscopic sterilization techniques with conventional methods using clinical and hemato-biochemical parameters and parameters related to stress.

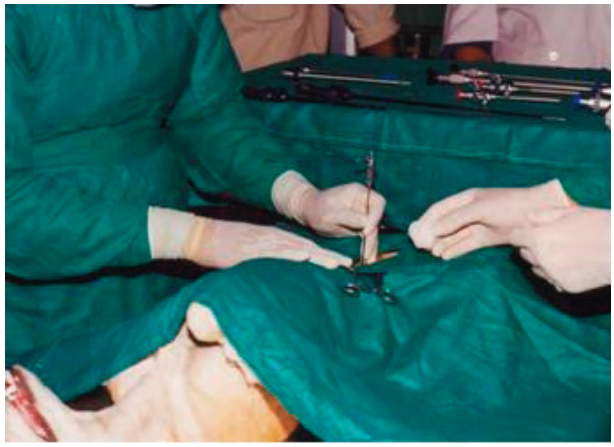

Figure 1. Making a small 0.5 cm skin incision at umbilicus and insertion of a Veres needle.

Materials and methods

The study was conducted on 10 clinically healthy, adult male dogs having body weights of 10 to 18.5 kg (14.10 ± 0.68) and of age 10 to 28 months (17.67 ± 1.22). The animals were randomly divided into 2 equal groups (I and II). In group I laparoscopic vasectomy by cauterization and cutting of the vas deferens was performed and in group II conventional open castration by the pre-scrotal approach was done. After administration of anesthesia the animals were placed in dorsal recumbency and then placed in a Trendelenburg position for laparoscopic vasectomy.

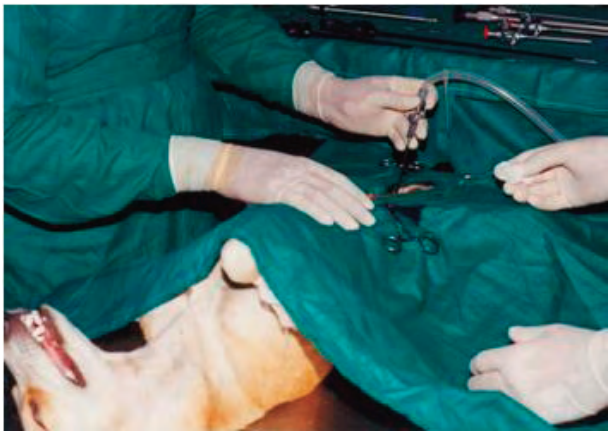

Figure 2. Insufflation of abdominal cavity with carbon dioxide gas.

A small 0.5 cm skin incision was made at the level of the umbilicus and a Veres needle was inserted (Figure 1). 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 (Figure 2). A 6 mm safety trocar and cannula unit was inserted into the abdominal cavity. A rigid type telescope (30 degree, 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 (Figure 3). The intraperitoneal organs along with the vas deferens were thoroughly visualized. The urinary bladder was visualized first by its characteristic tortuous structures of blood vessels. Then the vas deferens and spermatic artery-vein plexus were visualized (Figure 4). The vas deferens was identified by its characteristic ivory-colored, cord-like structure (Figure 5). Two ports of 6 mm size

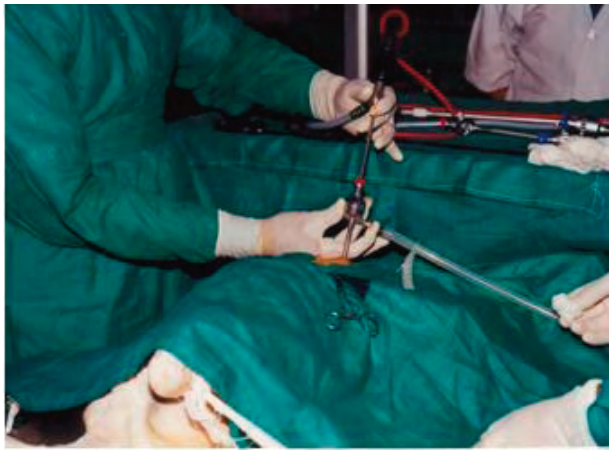

Figure 3. Insertion of telescope connected with light source and digital camera through cannula.

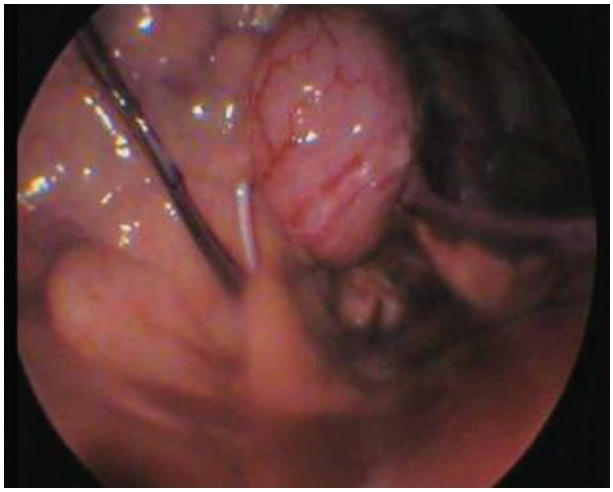

Figure 4. Visualization of urinary bladder, abdominal organs along with vas deferens and spermatic artery-vein plexus.

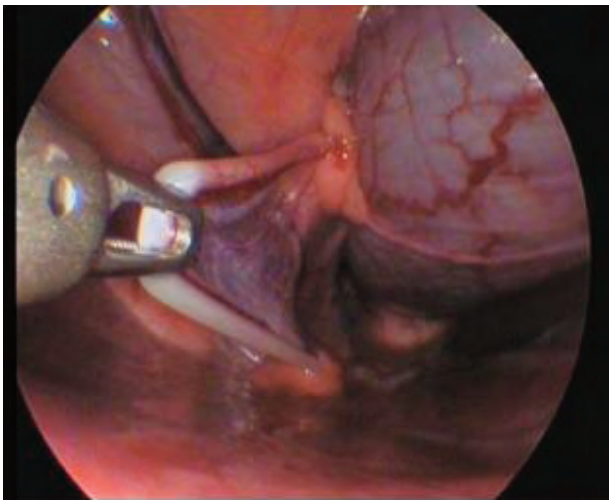

Figure 5. Vas deferens having characteristic ivory-colored, cord like structure.

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. The vas deferens was held by fenestered grasping forceps and the monopolar scissors were inserted through the opposite side port to cauterize the vas deferens and it was attached with an electrocautery unit (Figure 6). A 60 W monopolar current was used for cutting and cauterization. A piece of 2-3 cm of vas deferens was resected after

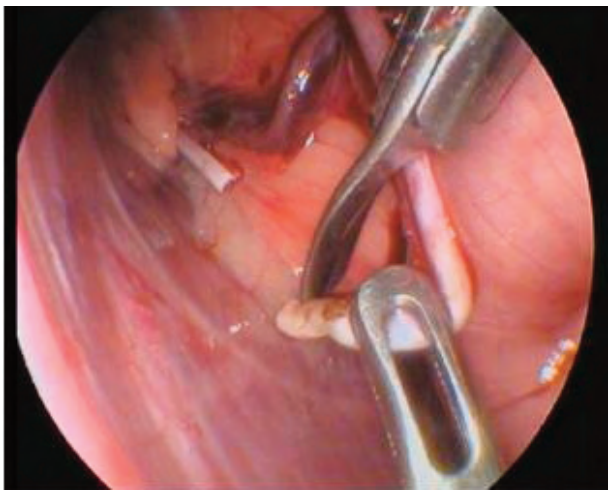

Figure 6. Holding of vas by grasping forceps and cutting and cauterization of vas deferens.

coagulation and removed through the cannula. The same procedure was repeated for the opposite vas deferens. In animals in group II conventional open castration by the standard pre-scrotal method was done. The animals were evaluated on the basis of following observations:

Intraoperative and postoperative observations

The operative techniques were evaluated based on flow rate and total utilization of carbon dioxide for each operation, instruments required, organ manipulation and maneuverability, intraoperative complications, and surgical operating time, which was defined as from the beginning of the first incision and up to the last skin suture. General behavior including discomfort and uneasiness, feeding habits, defecation and urination, and licking of the suture site. Each animal in group I was carefully monitored for complications like emphysema, port site herniation, bacterial peritonitis, ascites, and stitch abscess. The animals in group II were monitored for complications such as hemorrhage, wound dehiscence, infections, maggot infestations, and scrotal swelling.

Clinical observations

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

Hematological and biochemical observations

Blood smears were made for differential leukocyte count (DLC) using the standard procedure before the start of the operation, immediately after the completion of the operation and on days 1, 3, 5, and 7 after surgery. Heparinized blood was collected before the start of and immediately after the completion of the operation and on days 1, 3, 5, and 7 after surgery for estimation of alkaline phosphatase and acid phosphatase.

Estimation of oxidative stress

The PBS suspended RBCs were used to evaluate oxidative stress by estimating lipid peroxidation (LPO), catalase, superoxide dismutase (SOD), and reduced glutathione. The plasma samples were used to estimate the ceruloplasmin (acute phase proteins).

Hormonal estimation

The plasma samples were used to estimate the cortisol and testosterone hormone by radio-immunoassay using an RIA kit.

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

Results

Intraoperative and postoperative observations

The surgical phase of anesthesia in all the animals was achieved by administering xylazine and ketamine in combination. The postsurgical recovery from anesthesia in both the groups was smooth and uneventful. Establishment of capnoperitoneum (CP) in group I was easy and safe. The 10 mmHg pressure gradient was adequate to perform laparoscopic sterilization. The CO_2 flow rate of 2 L/min was also sufficient to maintain intra-abdominal pressure during surgery. For laparoscopic sterilization in group I 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 vas deferens. There was good exposure and visibility of the vas deferens and testicular vessels. Lifting of the vas by fenestered grasping forceps facilitated accuracy in cutting and cauterization. In group II, conventional open castration by pre-scrotal method was uneventful in each animal with adequate hemostasis. However, in all the animals swelling of the scrotum was observed. This was treated by administration of Meloxicam (MELONEX, Intas Pharmaceuticals Ltd., Ahmedabad, India) 0.02 mg/kg body weight and Ceftrioxone (INTACEPH, Intas Pharmaceuticals Ltd.) 10 mg/kg body weight intramuscularly for 5 days. The laparoscopy group appeared quite alert and responsive throughout the postoperative observation period, whereas in group II the animals were restless and were licking at the surgical wound site due to swelling and pain.

Clinical observations

The respiration rate (breaths/min), heart rate (beats/min), and rectal temperature (°F) recorded in all the animals of both groups are presented in Table 1. 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 both groups. No significant difference ($P > 0.05$) was observed in respiration rate when compared between the groups. Heart rate decreased non-significantly ($P > 0.05$) immediately after the operation in both groups. Preoperative as well as postoperative mean rectal temperature recorded in animals of group I remained within the normal limits. In contrast, the mean rectal temperature recorded after conventional open castration (group II) was significantly higher ($P < 0.05$) on the 1st and 3rd postoperative days than the preoperative base values. It returned to base values on the 5^{th} postoperative day.

Hematological and biochemical observations

Mean ± SE value of differential leukocyte count (DLC), alkaline phosphatase (ALP), and acid phosphatase (ACP) are presented in Table 2. DLC of both the groups revealed a significant increase ($P < 0.05$) of neutrophils on the 3rd postoperative day and comparative lymphopenia. No significant change ($P > 0.05$) was observed when comparisons were made between the groups at different time intervals. A significant increase ($P < 0.01$) in ALP level was observed on the 3rd postoperative day in both groups. Later, it decreased and returned to base values on day

Table 1. Mean ± SE value of respiration rate, heart rate and rectal temperature recorded at different time intervals.

Parameters	Groups	Before operation	Immediately after operation	Day 1 P.O.	Day 3 P.O.	Day 5 P.O.	Day 7 P. O.
Respiration Rate (beats/min)	I	25.20 ± 1.28[a]	18.80 ± 0.58[b]	25.40 ± 0.51	24.40 ± 0.75	24.40 ± 0.93	22.60 ± 0.81
	II	26.20 ± 1.28[b]	20.80 ± 0.66[a]	23.80 ± 1.53	26.00 ± 1.10	22.40 ± 1.03	24.80 ± 0.80
Heart Rate (beats/min)	I	117.80 ± 5.00	110.60 ± 5.04	116.60 ± 3.23	116.60 ± 4.01	118.20 ± 4.08	115.20 ± 4.27
	II	106.40 ± 8.45	96.80 ± 6.98	109.40 ± 6.23	111.20 ± 6.83	105.00 ± 4.51	107.00 ± 5.93
Rectal Temperature (°F)	I	101.30 ± 0.25	101.10 ± 0.24	101.10 ± 0.40	101.10 ± 0.19	100.50 ± 0.16	100.60 ± 0.29
	II	101.20 ± 0.34[a]	100.90 ± 0.29	102.10 ± 0.29[b]	102.10 ± 0.19[b]	101.30 ± 0.30	101.20 ± 0.25

Means with different superscripts (a, b) differ significantly (P < 0.05) within the group

Table 2. Mean ± SE values of alkaline phosphatase (U/L) and acid phosphatase (U/L) recorded at different time intervals.

Parameters	Groups	Before operation	Immediately after operation	Day 1 P.O.	Day 3 P.O.	Day 5 P.O.	Day 7 P. O.
Alkaline phosphatase (U/L)	I	11.37 ± 2.33[a]	11.93 ± 1.68[a]	10.89 ± 1.38[a]	32.87 ± 3.97[b]	15.35 ± 4.76[a]	9.77 ± 0.50[a]
	II	12.19 ± 1.98[a]	12.74 ± 1.00[a]	13.49 ± 1.03[a]	38.59 ± 0.90[b]	15.08 ± 1.48[a]	12.22 ± 1.17[a]
Acid phosphatase (U/L)	I	1.65 ± 0.03[a]	1.69 ± 0.03[a]	1.83 ± 0.02[b]	1.73 ± 0.03[a]	1.72 ± 0.02[a]	1.66 ± 0.03[a]
	II	1.78 ± 0.05[a]	1.79 ± 0.02[a]	1.83 ± 0.03[a]	1.96 ± 0.02[b]	1.79 ± 0.03[a]	1.74 ± 0.04[a]

Means with different superscripts (a, b) differ significantly (P < 0.05) within the group at different time intervals

7. When compared between the groups a significant increase (P < 0.05) was observed on day 1 in group II as compared to group I. A significant increase (P < 0.05) in the level of plasma ACP was observed on the 1st and 3rd postoperative days. However, no significant difference between the groups was observed at different time intervals.

Estimation of oxidative stress

The mean ± SE values of lipid peroxidation, catalase, superoxide dismutase, reduced glutathione and ceruloplasmin are presented in Table 3. No significant change in lipid peroxidation (LPO) values was observed at different time intervals in either group and the values returned to base values on day 7. A significant increase (P < 0.01) in catalase was observed on the 1st and 3rd postoperative days in both groups. Thereafter, the values of catalase enzymes decreased and returned to base line values on day 7 postoperatively. Values of superoxide dismutase (SOD) enzyme decreased significantly (P < 0.05) from the base value immediately after the operation in both groups. The values increased on subsequent intervals and reached the base values on day 5 postoperatively. A significant increase (P < 0.05) in reduced glutathione was recorded up to day 1. Later, the values decreased and returned to base line values on day 7 postoperatively. A comparison between the groups did not reveal any significant change (P > 0.05) in this enzyme between the groups at different time intervals. No significant change (P > 0.05) was observed on any of the postoperative days in the acute phase proteins (ceruloplasmin). However, a transient mild elevation (P > 0.05) of this protein was observed up to the 7th postoperative day in both groups.

Hormonal estimation

Mean ± SE of plasma cortisol and testosterone values of the groups are shown in Table 4. The

Table 3. Mean ± SE values of lipid peroxidation, catalase, superoxide dismutase, reduced glutathione and ceruplasmin recorded at different time intervals

Parameters	Groups	Before operation	Immediately after operation	Day 1 P.O.	Day 3 P.O.	Day 5 P.O.	Day 7 P. O.
Lipid peroxidation (LPO)	I	4.92 ± 0.37	6.10 ± 0.67	6.77 ± 1.33	5.74 ± 0.97	5.23 ± 0.76	4.72 ± 0.83
	II	4.51 ± 0.67	6.00 ± 0.37	6.05 ± 1.07	5.74 ± 1.33	4.92 ± 1.15	4.51 ± 1.01
Catalase	I	573.80 ± 164.2^{a}	1031.11 ± 137.2^{b}	1555.88 ± 273.3^{b}	960.01 ± 126.5^{b}	573.80 ± 51.1^{a}	386.21 ± 30.2^{a}
	II	540.69 ± 102.32^{a}	1346.22 ± 241.50^{b}	1975.20 ± 391.07^{b}	948.98 ± 80.71^{b}	648..09 ± 71.07^{a}	507.59 ± 86.18^{a}
Superoxide dismutase (SOD)	I	0.11 ± 0.01^{a}	0.07 ± 0.01^{b}	0.07 ± 0.02^{b}	0.10 ± 0.02^{a}	0.11 ± 0.01^{a}	0.13. ± 0.01^{a}
	II	0.06 ± 0.01^{a}	0.04 ± 0.00^{b}	0.03 ± 0.00^{b}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05. ± 0.00^{a}
Reduced glutathione	I	0.26 ± 0.02^{a}	0.34 ± 0.02^{b}	0.32 ± 0.01^{b}	0.30 ± 0.01^{b}	0.28 ± 0.01^{a}	0.27 ± 0.02^{a}
	II	0.15 ± 0.02^{a}	0.31 ± 0.05^{b}	0.56 ± 0.17^{b}	0.33 ± 0.03^{b}	0.24 ± 0.03^{b}	0.20 ± 0.02^{a}
Ceruplasmin	I	0.32 ± 0.02	0.36 ± 0.03	0.42 ± 0.07	0.33 ± 0.05	0.31 ± 0.04	0.30 ± 0.02
	II	0.25 ± 0.02	0.32 ± 0.04	0.29 ± 0.02	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.03

Means with different superscripts (a, b) differ significantly ($P < 0.05$) within the group
Units of different parameters estimated: 1. Lipid peroxidation (LPO) (nM/mL packed RBCs), 2. Catalase (nM H_2O_2 utilized/min/mL packed RBCs), 3. Superoxide dismutase (SOD) (mg/inhibition of 50% auto-oxidation of pyrogallol), 4. Reduced glutathione (mM/mL packed RBCs), 5. Ceruplasmin (g/L)

Table 4. Mean ± SE values of cortisol (nM/L) and testosterone (ng/mL) recorded at different time intervals.

Parameters	Groups	Before operation	Immediately after operation	Day 1 P.O.	Day 3 P.O.	Day 5 P.O.	Day 7 P. O.
Cortisol (nM/L)	I	25.59 ± 17.37^{a}	46.85 ± 16.39^{b}	18.99 ± 9.29^{a}	41.29 ± 16.48^{b}	29.88 ± 13.19^{a}	17.40 ± 8.45^{a}
	II	22.87 ± 4.89^{a}	64.03 ± 18.68^{b}	50.71 ± 23.24^{b}	21.81 ± 2.88^{a}	15.79 ± 1.83^{a}	18.34 ± 7.73^{a}
Testosterone (ng/mL)	I	0.418 ± 0.05^{a}	0.321 ± 0.03^{a}	0.396 ± 0.02^{a}	0.438 ± 0.09^{a}	0.369 ± 0.03^{a}	0.372 ± 0.04^{a}
	II	0.513 ± 0.08^{a}	0.028 ± 0.01^{b}	0.003 ± 0.01^{b}	0.002 ± 0.01^{b}	0.002 ± 0.00^{b}	0.001 ± 0.00^{b}

Means with different superscripts (a, b) differ significantly ($P < 0.05$) within the group at different time intervals

preoperative plasma cortisol level did not show any significant change between the groups. A significant increase ($P < 0.01$) in cortisol level was observed immediately after the operation in group II. In subsequent intervals the cortisol level decreased and returned to base values on day 7 in both groups. Preoperative testosterone values of the groups did not show any significant difference ($P > 0.05$). However, in group II a significant decrease ($P < 0.01$) in testosterone level was observed immediately after the operation and thereafter the values showed a gradual decrease ($P > 0.05$) up to the 7th postoperative day. No significant change ($P > 0.05$) in testosterone was observed at different postoperative time intervals in group I.

Discussion

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 (1). Laparoscopy has numerous advantages over traditional celiotomy techniques including decrease postoperative stress, faster recovery periods, decreased hospitalization, improved cosmesis, and improved visualization of abdominal organs (2). Control of pain and stress, being the beneficial aspects of minimally invasive surgery, are important factors for treatment of veterinary surgical patients. Researchers are continually looking for more progressive and less stressful surgical ways for sterilization in dogs. In the adult dog, intra-abdominal bilateral occlusion of the ductus deferens using laparoscopy and electrocoagulation resulted in the immediate absence of motile spermatozoa from the ejaculate in the long term without increasing the occurrence of variant postsurgical effects (3).

In this study laparoscopic sterilization in male dogs was compared with the conventional open method of castration. The laparoscopic and conventional surgical procedures were conducted under xylazine and ketamine general anesthesia. Induction as well as recovery from general anesthesia was smooth and uneventful in all the animals as also reported by Wildt et al. (1) using this combination of anesthesia for direct observation of internal organs of dogs and for the sterilization of male dogs by laparoscopic occlusion of the ductus deferens.

During laparoscopic sterilization of the animals in group I CO_2 pneumoperitoneum or capnoperitoneum (CP) was established at 10 mm of Hg pressure gradients intra-abdominally. The initial flow rate of carbon dioxide at 2 L/min was sufficient to achieve capnoperitoneum. This pressure and flow rate provided adequate inflation and excellent working space as also observed by Dharmaceelan et al. (4). Three ports were adequate to conduct the laparoscopic bilateral vasectomy in group I. Two 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 and the remaining one was at the umbilical site for insertion of the telescope as also reported by Wildt et al. (3). The urinary bladder was visualized first with the introduction of the telescope into the abdominal cavity and 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 (4), laparoscopic occlusion of the ductus deferens in male dogs and cats (3), and laparoscopic vasectomy in male dogs (5). Proper fasting prior to surgery emptied the colon and urinary bladder and thereby facilitated proper visualization of the vas deferens and spermatic artery-vein plexus (5).

All the animals returned to their normal feeding habits within 8-10 h of surgery. Urination and defecation were normal up to the 7th postoperative day. The laparoscopy group appeared quite alert and responsive. However, in group II, postsurgical pain due to scrotal swelling in all the animals was a major concern. The behavior of the animals in group II was quite different from that in group I such as restlessness and licking at the surgical wound site due to pain. No infection was observed in any animal postoperatively.

In clinical observations no significant differences in physiological parameters such as heart rate, respiration rate, and 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 (6). In the conventional open castration group (group II) the mean rectal temperature showed a significant increase ($P < 0.05$) on the 1st and 3rd postoperative days but there was no significant change in respiration or heart rate. The change in rectal temperature might be attributed to surgical stress as a result of surgical trauma in group II. The intensity of trauma to tissues was higher in group II than in the laparoscopic group. In both groups, there was a non-significant decrease in respiration rate as well as in heart rate immediately after the operation attributable to the post-anesthetic effect of xylazine.

A significant increase ($P < 0.05$) in neutrophils and comparative lymphopenia was observed on the 3rd postoperative day in both groups of animals. There was no significant difference between the groups. The altered leukogram could be as the result of a release of endogenous glucocorticoids in response to tissue trauma and inflammation (7). In contrast, Earley and Crowe (8) reported that surgical castration did not affect any of the hematological parameters from day 0

to 3 after surgery and would indicate that the health of the animals was not compromised. The plasma ALP levels in animals were within the normal limits, but a significant increase ($P < 0.05$) in this enzyme was observed on the 3rd postoperative day in both groups. The elevation in plasma ALP after laparoscopy in group I may be attributed to tissue injury as a result of ischemia reperfusion induced oxidative stress in the liver and kidney following capnoperitoneum (9). In group II marked plasma ALP activity on the 1st and 3rd postoperative days might be attributed to the release of endogenous corticosteroids in response to profound tissue injury and stress (10). Acid phosphatase (ACP) activity was reported to be derived from lysosomal compartment of cells, predominantly in bone and to some extent in other cells like platelets, erythrocyte, and spleen (11). A significant increase in plasma ACP was observed on the 1st and 3rd postoperative days, but there was no significant difference between 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.

Estimations of oxidative stress indices revealed no significant change in LPO in groups I or II; however, a mild transient increase in LPO was observed after the operation up to the 3rd postoperative day. The increased LPO might be a result of decreased SOD activity after the operation. The values returned to base level on the 7th postoperative day in both groups. The catalase activity significantly increased on the 1st and 3rd postoperative days and returned to the base line value on the 7th postoperative day. This showed that the production of catalase as an antioxidant enzyme against reactive oxygen species (ROS) was enhanced by increased lipid peroxidation. The increased catalase production acts against oxygen free radical molecules, which were produced when cell damage occurred due to lipid peroxidation of cell membranes. Kumaraguruparan et al. (12) also reported that an increase in LPO accompanied by an enhanced antioxidant status provides the first line of defense against ROS induced damage. The increase in catalase level indicates that these enzymes were not utilized against H_2O_2 because the production of these radicals from superoxide radicals fell due to decreased activity of SOD in both groups.

The SOD activity was significantly lower in both groups up to the 3rd postoperative day, but it reached the base values on the 5th postoperative day. The decreased SOD after the operation was attributed to decreased LPO in these groups. Fridovich (13) reported that the increased activity of SOD dismutes the superoxides and resulted in the generation of H_2O_2, which is decomposed by CAT into H_2O and O_2. A significant increase in the reduced glutathione was observed immediately after the operation and on the 1st postoperative day. Later the values decreased and returned to the base line values on the 5th day postoperatively. Bisla et al. (14) also reported that a significant increase in malondialdehyde (MDA), a main indicator of lipid peroxidation, GSH, and oxidative stress factor (OSF) occurred after herniorrhaphy in buffaloes. Enhanced expression of GSH-dependent enzyme GPx has been documented to inhibit ROS-induced apoptosis in human breast cancer cell lines (15). Hafeman et al. (16) reported that GPx played a crucial role in preventing membranes from peroxide damage induced by lipid peroxides. This study showed that enhanced production of reduced glutathione occurred in both groups in order to prevent oxidative stress due to different surgical procedures performed on these animals.

The ceruloplasmin level increased non-significantly ($P > 0.05$) after surgery and the level in plasma remained elevated non-significantly up to the 7th postoperative day in both groups. Conner et al. (17) also reported increased ceruloplasmin levels in plasma after surgical trauma due to acute phase inflammatory process in the dog. Solter et al. (18) reported that haptoglobin and ceruloplasmin have greater sensitivity as determinants of inflammation in dogs. APPs level increases in the circulation after surgery and its associated tissue damage are particularly associated with inflammation from whatever cause (19).

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 (20). In the present study, a significant increase ($P < 0.01$) in plasma cortisol was observed immediately after the operation in group II, whereas in group I a non-significant increase

($P > 0.05$) was observed. It might be attributed to the pain inflicted at the time of surgery and inflammation following surgery. Kehlet (21) reported that following surgery inflammation and pain due to trauma are major inducers of cortisol secretion. When compared between the 2 groups a significant increase ($P < 0.01$) in plasmal cortisol was observed immediately after the operation in group II. Marcovich et al. (22) reported a higher cortisol level at 4 h, whereas the peak cortisol level after laparoscopy in dog was observed at 2 h by Hancock et al. (23).

Testosterone is a male reproductive hormone often used to evaluate the reproductive status of the animal. The testes are the major source of this hormone, although it has been secreted in very minute quantities from other source like the adrenal cortex. Due to the source specificity, testosterone estimation has been indicated to assess the effects of surgical techniques in the present study. In group I, no significant change ($P > 0.05$) in testosterone was observed at different postoperative time intervals. It was attributed to bilateral vasectomy, which does not exert a remarkable effect on the steroidogenic functionality of the testicle as also reported by Batista et al. (24). In group II, a significant decrease ($P < 0.01$) in plasma testosterone was observed immediately after the operation up to the 7th postoperative day. Ortega-Pacheco et al. (25) reported that neutering of male dogs by the Burdizzo castrator causes slight but non-significant increases in testosterone concentration after gonadorelin challenge. In group II, the significant decrease in plasma testosterone is attributed to the removal of its major source. Niu et al. (26) reported that following castration the serum concentration of testosterone decreased rapidly in 2 days. Ortega-Pacheco et al. (25) also reported a significant decrease ($P < 0.001$) in serum testosterone after surgical castration in dogs.

References

1. Wildt, D.E., Kinney, G.M., Seager, S.W.J.: Laparoscopy for direct observation of internal organs of the domestic cat and dog. Am. J. Vet. Res., 1977; 38: 1429-1432.
2. Gyr, T., Ghezzi, F., Arslanagic, S., Leidi, L., Pastorelli, G., Franchi, M.: Minimal invasive laparoscopic hysterectomy with ultrasonic scalpel. Am. J. Surg., 2001; 181: 516-519.
3. 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.
4. 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.
5. Silva, L.D., Onclin, K., Donnay, I., Verstegen, J.P.: Laparoscopic vasectomy in the male dog. J. Reprod. Fertil. Suppl., 1993; 47: 399-401.
6. 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.
7. Latimer, K.S.: Leukocytes in health and disease. In: Ettinger, S.J., Feldman, E.C. Eds., Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat. 4th edn., W.B. Saunders, Philadelphia. 1995; 1892-1946.
8. Earley, B., Crowe, M.A.: Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci., 2002; 80: 1044-1052.
9. 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.
10. Johnson, S.E., Sherding, R.G.: Diseases of liver and biliary tract. In: Brichard, S.J., Sherding, R.G. Eds., Saunders Manual of Small Animal Practice. W.B. Saunders Co., Philadelphia. 1999; 824-873.
11. Rosol, T.J., Capen, C.C.: Calcium regulating hormones and diseases of abnormal mineral metabolism. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L. Eds., Clinical Biochemistry of Domestic Animals. Academic Press, London. 1997; 619-702.
12. Kumaraguruparan, R., Balachandran, C., Manohar, B.M., Nagini, S.: Altered oxidant-antioxidant profile in canine mammary tumours. Vet. Res. Commun., 2005; 29: 287-296.
13. Fridovich, I.: Free Radicals in Biology. In: Proyer, W.Q. Ed., Vol. 1, Academic Press, New York. 1978; 239.
14. Bisla, R.S., Jit Singh, Chawla, S.K., Krishnamurthy, D.: Assessment of ascorbic acid as an anti-oxidant in buffaloes during diaphragmatic herniorrhaphy. Indian J. Vet. Surg., 2004; 25: 94-97.
15. Gouazé, V., Mirault, M.E, Carpentier, S., Salvayre, R., Levade, T., Andrieu-Abadie, N.: Glutathione peroxidase-1 overexpression prevents ceramide production and partially inhibits apoptosis in doxorubicin-treated human breast carcinoma cells. Mol. Pharmacol., 2001; 60: 488-496.

16. Hafeman, D.G., Sunde, R.A., Hoekstra, W.G.: Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 1974; 104: 580-587.

17. Conner, J.G., Eckersall, P.D., Ferguson, J., Douglas, T.A.: Acute-phase response in the dog following surgical trauma. Res. Vet. Sci., 1988; 45: 107-110.

18. Solter, P.F., Hoffmann, W.E., Hungerford, L.L., Siegel, J.P., Denis, S.H., Dorner, J.L.: Haptoglobin and ceruloplasmin as determinants of inflammation in dogs. Am. J. Vet. Res., 1991; 52: 1738-1742.

19. Eckersall, P.D., Conner, J.G.: Bovine and canine acute phase proteins. Vet. Res. Commun., 1988; 12: 169-178.

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

21. Kehlet, H.: Neurohumoral response to surgery and pain in man. In: Bond, M.R., Charlton, J.E., Woolf, C.J. Eds., Proc. 6th World Congress on Pain. Elsevier, The Netherlands, Amsterdam. 1991; 35-40.

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. 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.

24. Batista, M., Prats, N., Calero, P., González, F., Cabrera, F., Medrano, A., Gracia, A.: Semen characteristics and plasma levels of testosterone after bilateral vasectomy in bucks. Reprod. Domest. Anim., 2002; 37: 375-378.

25. Ortega-Pacheco, A., Bolio-González, M.E., Colin-Flores, R.F., Sauri-Arceo, C.H., Guttiérrez-Blanco, E., Jiménez-Coello, M., Linde Forsberg, C.: Evaluation of a Burdizzo castrator for neutering of dogs. Reprod. Domest. Anim., 2006; 41: 227-232.

26. Niu, Y.J., Ma, T.X., Zhang, J., Xu, Y., Han, R.F., Sun, G.: Androgen and prostatic stroma. Asian J. Androl., 2003; 5: 19-26.