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ORIGINAL ARTICLE

Laparoscopic Vasectomy *vs* Laparoscopic Sterilization in Dogs: A Comparison of Two Techniques

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ABSTRACT

Twelve clinically healthy, adult male dogs randomly equally divided into two groups (I and II). Animals of both the groups received xylazine-ketamine anesthesia. Laparoscopic bilateral vasectomy was performed in group I, whereas in animals of group II in addition to vasectomy, spermatic artery-vein plexus were clipped with titanium clips at a distance of 1 to 2 cm. Insufflation of abdominal cavity was achieved by CO_2 (2 liter/minute) at 10 mm Hg pressure gradient. Clinical observations revealed no significant changes. Differential leukocyte count (DLC) revealed significant neutrophilia and comparative lymphopenia on 3rd postoperative in both groups. Significant increase ($p < 0.05$) in plasma alkaline and acid phosphatase level was observed on day 3 postoperatively. Indices of oxidative stress *viz* lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), reduced glutathione activity and acute phase protein, ceruloplasmin level in plasma did not revealed any major significant changes but indicated that oxidative stress was more in group II animals. Plasma cortisol level increased significantly ($p < 0.01$) after operation and testosterone level showed gradual decrease ($p > 0.05$) up to 7th postoperative day in animals of group II. On the basis of the parameters studied, it can be concluded that capnoperitoneum at 10 mm Hg pressure gradient and CO_2 at the flow rate of 2 liter/minute provides optimum visualization of intra-abdominal organs and found suitable for laparoscopic sterilization in male dogs. The laparoscopic vasectomy alone in male dogs was found comparatively quick, less time consuming and can be successfully applied for mass sterilization program. Oxidative stress in laparoscopic vasectomy (group I) was less as compared to other group.

Keywords: Male dogs, Laparoscopic vasectomy, Sterilization, Oxidative stress, Lipid peroxidation, Catalase, Superoxide dismutase, Reduced glutathione, Acute phase proteins.

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INTRODUCTION

Laparoscopic surgery provides a wide field of its extensive application particularly in surgical sterilization of different animal species. Furthermore, high demographic urban and industrial area requires an effective animal birth control program, which can overcome the problem of hospitalization, postoperative complications and the overall cost reduction of operation.[1] Castration of male dogs by conventional open method have many disadvantages and postoperative complications, such as hemorrhage, wound dehiscence, infections, maggot infestations and scrotal swellings, etc. In a large scale animal birth control program, the conventional methods of sterilization requires 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 or laparoscopic surgery can revolutionize the entire program, as it needs only very small surgical wound, which usually needs no postoperative care or regular dressings. The laparoscopic surgery has advantages, like minimal invasiveness with maximum visibility, shorter surgical time, decreased postoperative discomfort and pain, less incidences of infection, uncomplicated healing with minimal scarring and minimal surgical morbidity.[2] It also avoids postoperative complications, such as wound dehiscence, herniation, etc. and reduces the surgical stress to animal as well as recurring cost of each surgery. Therefore, the present study was undertaken to compare laparoscopic vasectomy and laparoscopic sterilization, i.e. vasectomy plus clipping of spermatic artery vein plexus, using clinical, hematobiochemical parameters and parameters related to stress.

MATERIALS AND METHODS

The study was conducted on 12 clinically healthy, adult male dogs having body weights of 13.0 to 18.5 kg (15.10 ± 0.68) and of age 20 to 28 months (24.57 ± 1.56). The

animals were randomly divided into two equal groups (I and II). In group I, laparoscopic vasectomy by cauterization and cutting of vas deferens was performed. In group II, along with laparoscopic vasectomy as done in group I, clipping of spermatic artery vein plexus was also performed. The animals were premedicated with atropine sulphate at the dose rate of 0.04 mg/kg body weight subcutaneously. Fifteen minutes later xylazine hydrochloride (at the dose rate of 1.0 mg/kg body weight) and ketamine hydrochloride (at the dose rate of 10.0 mg/kg body weight) were administered intramuscularly. Depth of analgesia was monitored during the entire period of surgery. Incremental doses of ketamine may be given if required. After administration of anesthesia the animals were placed in dorsal recumbency having a Trendelenburg position.

A small 0.5 cm skin incision was made at the level of umbilicus. By grasping the skin around the incision by one hand, simultaneous insertion of Veress needle was done by other hand. Intraperitoneal placement was confirmed by injecting 5 ml of saline through the needle. Insufflation of abdominal cavity was done by carbon dioxide gas at the rate of 2 l/min with pressure gradient of 10 mm Hg in both the groups. After attaining a sufficient pneumoperitoneum, Veres needle was removed and a 6 mm safety trocar and cannula unit was inserted into the abdominal cavity. A rigid type telescope (30º, 5 mm in diameter, Frontline Co, Germany) connected with light source (40 W, Halogen lamp) and digital camera was then introduced through cannula. Two ports of 6 mm size were created using 6 mm trocar-cannula unit under the guidance of telescope distal to the laparoscope insertion site and 4 to 6 cm bilaterally from the ventral midline. Through these ports, the operative instruments were inserted for surgical procedures. The intraperitoneal organs along with vas deferens were thoroughly visualized. The urinary bladder was visualized first by its characteristics tortuous structures of blood vessels. Then, the vas deferens and spermatic artery-vein plexus were visualized. The vas deferens was identified by its characteristic ivory-colored, cord-like structure (Fig. 1). Each vas deferens was observed at the site where both of them joined dorsal to the bladder, and they were easily traced to the point where they enter the abdominal cavity at the inguinal ring. The vas deferens was held by fenestered grasping forceps, inserted through the same side port (Fig. 2). The monopolar scissors was inserted through the opposite side port to cauterize the vas deferens and attached it with electrocautery unit. A 60 W monopolar current was used for cutting and cauterization. A piece of 2 to 3 cm of vas deferens was resected after coagulation and removed through the cannula (Fig. 3). The same procedure was repeated for the opposite vas deferens. In animals of group II, the procedure for vas deferens was repeated as mentioned in group I. After resection and removal of vas deferens, the spermatic artery-vein plexus were identified. Unlike in the scrotum, the vas deferens within the pelvic cavity was not associated with the spermatic artery-vein plexus that courses laterally along the dorsolateral portion of the abdominal wall. Clipping was done after holding these vessels by fenestered grasping forceps. The vessels were clipped by applying two titanium clips at a distance of 1 to 2 cm between them by using clip applicator (Fig. 4). The same procedure was repeated to the opposite one.

After completion of the procedure the carbon dioxide gas was removed and the port wounds were sutured with single interrupted mattress suture pattern using nylon. All the wounds were cleaned and dressed regularly at the portal and incisional site with povidone iodine and antiseptic cream. Dressing was done in the morning after recording the clinical parameters. Up to 7th postoperative day they were closely observed away from their sight, for any behavioral changes due to surgery.

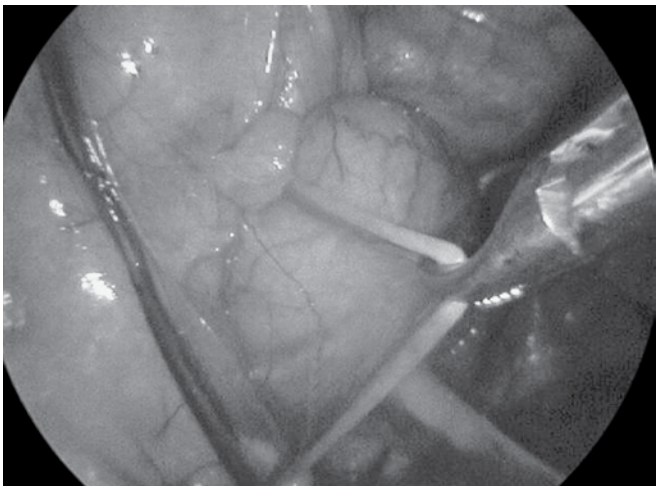

Fig. 1: Identification of the vas deferens by its characteristic ivory-colored, cord-like structure

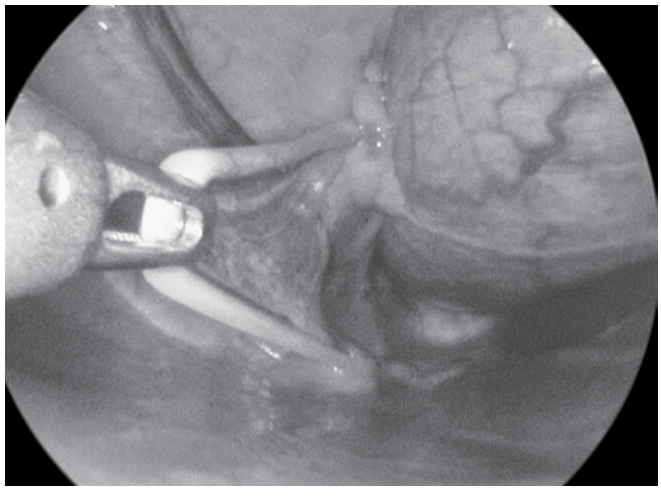

Fig. 2: Holding of the vas deferens by fenestrated grasping forceps in animals of group I

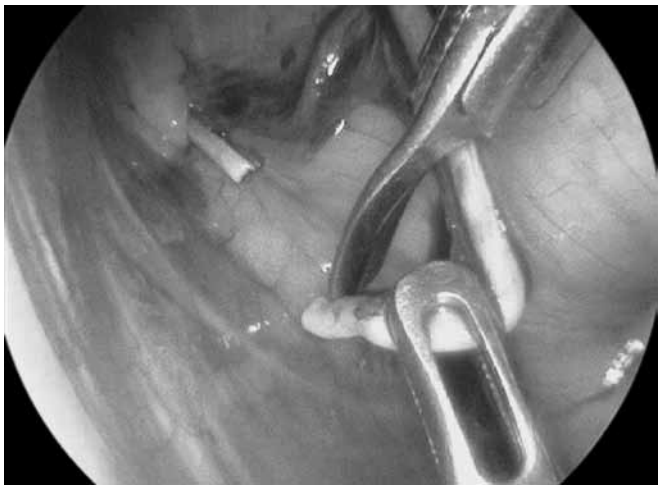

Fig. 3: A piece of 2 to 3 cm of vas deferens was resected after coagulation and removed through the cannula in group I

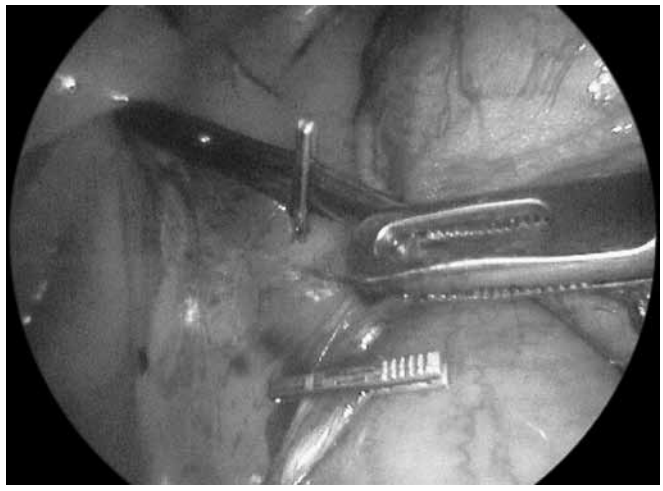

Fig. 4: After resection of vas deferens, the spermatic artery vein plexus was clipped by applying two titanium clips at a distance of 1 to 2 cm, using clip applicator in animals of group II

Intraoperative and Postoperative Observations

The two operative techniques were evaluated based on flow rate and total utilization of carbon dioxide for each operation, instrument required, organ manipulation and maneuverability, intraoperative complications and surgical time which was defined as from the beginning of first incision and up to the last skin suture. General behavior, including discomfort and uneasiness, feeding habits, defecation and urination, licking of the suture site, was observed up to 7th postoperative day. Each animal 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 (ºF) were recorded before start of operation, immediately after completion of operation and on days 1, 3, 5 and 7 after surgery.

Hematobiochemical Observations

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

Estimation of Oxidative Stress

The phosphate-buffered saline (PBS) suspended red blood cells (RBCs) were used to evaluate oxidative stress by estimating lipid peroxidation (LPO),[4] catalase (CAT),[5] superoxide dismutase (SOD)[6] and reduced glutathione.[7] The plasma samples were used to estimate the ceruloplasmin (acute phase proteins).[8]

Hormonal Estimation

The plasma samples were used to estimate the cortisol and testosterone hormone by radioimmunoassay (RIA) using RIA kit.[9]

The data were subjected to two-way analysis of variance (ANOVA) and the mean values of different time interval were compared with base level using paired 't' test.[10]

RESULTS

Intraoperative and Postoperative Observations

Surgical phase of anesthesia in all the animals were achieved by administering xylazine and ketamine combination. In both the groups no additional anesthesia was required in any animal during entire surgical procedure. The postsurgical recovery from anesthesia in both the groups was smooth and uneventful. Establishment of capnoperitoneum (CP) in each animal of both groups was found easy and safe. The CP was established at 10 mm Hg pressure gradient. This pressure was found adequate to perform laparoscopic surgery in the animals of both the groups. The CO_2 flow rate of 2 l/minute was also found sufficient to maintain intra-abdominal pressure during surgery. The total utilization of CO_2 gas for laparoscopic sterilization in group II (16.00 ± 0.78), which was significantly higher ($p < 0.01$) than animals of group I (8.04 ± 0.33).

For the laparoscopic sterilization, three ports were found sufficient to conduct the sterilization procedure but, the port size were different. In group I, three ports of 6 mm size were required whereas, in group II, one 6 mm size port for insertion of telescope (5 mm) and two 11 mm size ports for clip applicator (10 mm) were required. In group II, endoclips

were applied to the spermatic artery-vein plexus by using clip applicator. Two ports at paramedian site, either side of inguinal region, 4 to 6 cm lateral to mid-ventral line was found easy for laparoscopic sterilization. In all the animals of both groups monopolar coagulation current of 60 W was used and found effective for electrocautery as well as coagulation of the line of cutting of vas deferens. The visualization of vas deferens was better with trendelenburg position of the animals. All the animals were closely monitored for effective hemostasis before the final withdrawal of telescope.

Clinical Observations

All the animals were recovered smoothly from anesthesia. Return to normal appetite within 8 to 10 hours after recovery from anesthesia was observed in all animals. Urination and defecation were normal throughout the observation period. No significant change ($p > 0.05$) in respiration rate, heart rate and rectal temperature was observed. The values remained within the normal limits, without any significant change ($p > 0.05$) from base values at any postoperative time intervals (Table 1).

Hematobiochemical Observations

Differential leukocyte count of both the groups revealed a significant increase ($p < 0.05$) in neutrophils on 3rd postoperative day with comparative lymphopenia. No significant change ($p > 0.05$) was observed when comparisons were made between the groups at different time intervals. Significant increase ($p < 0.05$) in alkaline phosphatase (ALP) level was observed on 3rd postoperative day in animals of both the groups. Later on, it reduced and returned to base values on day 7 (Table 2). The acid phosphatase (ACP) level showed a significant increase ($p < 0.05$) on 1st and 3rd postoperative days (*see* Table 2).

Estimation of Oxidative Stress

The mean ± SE values of lipid peroxidation, catalase, superoxide dismutase reduced glutathione, ceruloplasmin are presented in Table 3. There was a mild, transient nonsignificant increase ($p > 0.05$) in LPO values after operation up to 3rd postoperative day in both the groups and it returned to base values on day 7. A significant increase ($p < 0.01$) in catalase was observed immediately after operation and on 1st and 3rd postoperative days in both the groups. Thereafter, the values of catalase enzymes decreased and returned to base line values on day 7 postoperatively. Values of SOD enzyme decreased nonsignificantly ($p > 0.05$) upto 3rd postoperative day in both the groups. The values increased on subsequent intervals and reached to base values on day 7 postoperatively in both the groups. A significant increase ($p < 0.05$) in reduced glutathione values upto day 1 was observed in both the groups. Later on, the values decreased and returned to base line values on day 7 postoperatively. No significant change ($p > 0.05$) was observed in ceruloplasmin level at any postoperative days. However, transient mild elevation ($p > 0.05$) of this protein was observed up to 7th postoperative day in both the groups.

Hormonal Estimation

The mean ± SE values of cortisol and testosterone are presented in Table 4. A nonsignificant increase ($p > 0.05$) in cortisol values was observed immediately after operation in group I. However, significant increase ($p < 0.01$) in cortisol level was observed immediately after operation in group II. On subsequent intervals, the cortisol level reduced and returned to base values on day 7 in both the groups. No significant change ($p > 0.05$) in testosterone was observed at different postoperative time intervals in group I. In group II, significant decrease ($p < 0.01$) in testosterone level was observed immediately after operation and there after the values showed gradual decrease ($p > 0.05$) up to 7th postoperative day.

DISCUSSION

The laparoscopic surgical techniques in both human and veterinary medicine have grown tremendously. Laparoscopy has been used to the routine diagnostic and therapeutic methods used especially in small animals. Therefore at present, laparoscopes provide a true panoramatic picture of the observed cavity. In addition to that, they can magnify to a certain degree, what makes visualization more precise and the picture brighter. For this reason, at using the laparoscopic techniques impairment of tissues hardly occurs. Since,

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 PO*	*Day 3 PO*	*Day 5 PO*	*Day 7 PO*
Respiration rate (breaths/min)	I	24.20 ± 1.28	18.80 ± 0.58	25.40 ± 0.51	24.40 ± 0.75	24.40 ± 0.93	22.60 ± 0.81
	II	26.00 ± 0.89	20.60 ± 0.68	24.40 ± 0.93	24.80 ± 1.66	24.60 ± 1.57	22.60 ± 1.03
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	115.40 ± 8.78	110.40 ± 7.39	108.40 ± 7.71	109.40 ± 6.37	112.00 ± 6.32	115.60 ± 5.78
Rectal temperature (ºF)	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	101.30 ± 0.41	101.20 ± 0.25	101.50 ± 0.35	101.10 ± 0.29	100.90 ± 0.29	101.00 ± 0.16

an operator can see details of the organ surface (structures about 1.0 mm and less), he can avoid the blood sinus and so prevent the undesirable bleeding. The laparoscopy requires minor surgical intervention and it provides the only available practical means of making repeated direct observation of abdominal viscera.[11] 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 way for sterilization in dogs. In the adult dog, intra-abdominal bilateral occlusion of ductus deferens using laparoscopy and electrocoagulation resulted in the immediate absence of motile spermatozoa from the ejaculate in long-term without increasing the occurrence of variant postsurgical effects.[12]

In the present study, the laparoscopic procedure was conducted under xylazine and ketamine general anesthesia. Both, induction as well as recovery from general anesthesia was smooth and uneventful in all the animals. Wildt et al[11] used this combination of anesthesia for direct observation of internal organs of dogs using laparoscopy. They found that induction was more rapid (5 minutes or less) in the dog. Drug tolerance was good with this combination even following frequent administrations requiring serial laparoscopy. Wildt et al[12] successfully used this combination of anesthesia for the sterilization of male dog by laparoscopic occlusion of the ductus deferens.

Intraoperative and Postoperative Observations

During laparoscopic sterilization in animals of groups I and II, CO_2 pneumoperitoneum or capnoperitoneum was established at 10 mm Hg pressure gradients intra-abdominally. The pressure gradient of 10 mm Hg and higher is required to conduct a laparoscopic surgery and has been reported by other workers in recent literatures.[13-15] The initial flow rate of carbon dioxide at 2 l/minute was found sufficient to achieve capnoperitoneum. Subsequently, after trocarization, the capnoperitoneum maintained by inflation of CO_2 at a flow rate of 2 l/minute. This pressure and flow rate provided adequate inflation and excellent working space. Maintenance of flow of CO_2 compensated the loss of CO_2 through the various ports during surgery. The findings

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 PO*	*Day 3 PO*	*Day 5 PO*	*Day 7 PO*
Alkaline phosphatase (U/L)	I	11.37 ± 2.33	11.93 ± 1.68	10.89 ± 1.38	32.87 ± 3.97**	15.35 ± 4.76	9.77 ± 0.50
	II	9.71 ± 0.73	10.61 ± 0.58	9.87 ± 0.18	30.15 ± 2.37**	11.62 ± 0.50	10.66 ± 0.54
Acid phosphatase (U/L)	I	1.65 ± 0.03	1.69 ± 0.03	1.83 ± 0.02*	1.73 ± 0.03	1.72 ± 0.02	1.66 ± 0.03
	II	1.78 ± 0.04	1.80 ± 0.04	1.83 ± 0.04*	1.82 ± 0.03	1.67 ± 0.06	1.70 ± 0.03

*Differ significantly ($p < 0.05$) from base values (before operation); **Differ significantly ($p < 0.01$) from base values (before operation)

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

Parameters	*Groups*	*Before operation*	*Immediately after operation*	*Day 1 PO*	*Day 3 PO*	*Day 5 PO*	*Day 7 PO*
Lipid peroxidation (nM/ml packed RBCs)	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	5.32 ± 0.22	5.74 ± 0.24	6.26 ± 0.17	6.00 ± 0.26	5.33 ± 0.30	4.87 ± 0.27
Catalase (nM H_2O_2 utilized/min/ml packed RBCs)	I	573.80 ± 164.2	1031.11 ± 137.2^{**a}	1555.88 ± 273.3^{**a}	960.01 ± 126.5*	573.80 ± 51.1	486.21 ± 30.2
	II	794.43d ± 164.2	1324.16 ± 137.2^{**b}	1246.91 ± 273.3^{**b}	1004.11 ± 126.5*	805.53 ± 51.1	617.94 ± 30.2
Superoxide dismutase (mg/ inhibition of 50% auto-oxidation of pyrogallol)	I	0.11 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.01
	II	0.08 ± 0.00	0.05 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
Reduced glutathione (mM/ml packed RBCs)	I	0.26 ± 0.02	0.28 ± 0.02	0.34 ± 0.01*	0.30 ± 0.01	0.28 ± 0.01	0.27 ± 0.02
	II	0.20 ± 0.03	0.26 ± 0.03	0.29 ± 0.03	0.29 ± 0.02	0.25 ± 0.03	0.21 ± 0.03
Ceruloplasmin (gm/liter)	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	0.30 ± 0.03	0.35 ± 0.03	0.37 ± 0.02	0.38 ± 0.03	0.34 ± 0.02	0.31 ± 0.03

Means with different superscripts (a, b) differ significantly ($p < 0.05$) within the group; *Differ significantly ($p < 0.05$) from base values (before operation); **Differ significantly ($p < 0.01$) from base values (before operation)

Table 4: Mean ± SE values of cortisol and testasterone recorded at different time intervals

Parameters	*Groups*	*Before operation*	*Immediately after operation*	*Day 1 PO*	*Day 3 PO*	*Day 5 PO*	*Day 7 PO*
Cortisol (nM/l)	I	25.59 ± 17.37	46.85 ± 16.39	31.99 ± 9.29	27.29 ± 16.48	27.88 ± 13.19	27.40 ± 8.45
	II	46.83 ± 4.99	129.08 ± 23.46**	59.41 ± 21.03	38.68b ± 12.78	23.55 ± 8.20	25.52 ± 5.36
Testosterone (ng/ml)	I	0.418 ± 0.05	0.321 ± 0.03	0.396 ± 0.08	0.438 ± 0.09	0.369 ± 0.03	0.372 ± 0.04
	II	0.437 ± 0.08	0.159 ± 0.03**	0.101 ± 0.02**	0.024 ± 0.00**	0.025 ± 0.01**	0.015 ± 0.01**

**Differ significantly ($p < 0.01$) from base values (before operation)

of the present study concurred with the observations of Dharmaceelan et al.[14] The total utilization of CO_2 during surgical procedures was recorded. Comparatively, less utilization of CO_2 was observed in group I because of less time taken to perform bilateral vasectomy. This method was comparatively simple, quick and easier than method of group II. In group II, utilization of CO_2 was significantly higher ($p < 0.01$) than that of group I, may be due to the longer operative time and may also be due to leakage of CO_2 during removal of resected tissue through 10 mm ports by 5 mm forceps.

The instruments used were differed in both groups. In group I, all the three ports were of 5 mm in size, two ports were created at left and right paramedian site distally to the telescope insertion site and 4 to 6 cm laterally at the inguinal regions and the rest one was at umblical site for insertion of telescope. In the group II, two ports were 10 mm in size and the rest one was 5 mm for telescopic insertion at the same sites as described in group I. These two 10 mm ports were needed mainly to insert the 10 mm clip applicator toward the contralateral spermatic artery-vein plexus and were converted into 5 mm size by applying adaptor for inserting fenestered grasping forceps to manipulate the vas deferens and testicular vessels for vasectomy as well as for clip application respectively. However, regarding the number of trocars and their respective sites, the present study concurred with the findings of Wildt et al.[12] The urinary bladder was visualized first with the introduction of telescope into the abdominal cavity and it was identified by its characteristics tortuous structures of blood vessels on it.

The 30º forward oblique, 5 mm rigid telescope used in this study covered sufficiently big exposure operative area in a single view without any remarkable loss of resolution and visibility. Most of the clinicians have reported the use of 5 mm telescope during ovariohysterectomy in dogs.[11,14,15] Laparoscopic occlusion of ductus deferens in male dogs and cats[12] and laparoscopic vasectomy in male dogs.[16] The separation of intra-abdominal organs from the ventral as well as lateral abdominal walls were adequate at 10 mm Hg pressure gradient intraperitoneally. Also, proper fasting prior to surgery emptied colon and urinary bladder[13] and thereby facilitated proper visualization of the vas deferens and spermatic artery-vein plexus.

The electrocautery with 60 W monopolar current revealed a good hemostatic measure in both groups. Rodgerson et al[17] found that application of coagulation current from monoplar electrocautery alone was sufficient for effective hemostasis for equine mesoovarium. However, before final withdrawal of telescope in all the animals of laparoscopy groups the resected sites were closely observed. The mean surgical operating time was significantly lower ($p < 0.05$) in group I as compared to group II. The decreased surgical operating time in group I was due to the reason that this technique was simple, quick and easier than other two groups. Wildt et al[12] reported the lower operative time required for bilateral vasectomy in dog. Increased surgical operative time in laparoscopic bilateral vasectomy with occlusion spermatic artery-vein plexus (group II) was due to additional time required for application of endoclips. All the animals were returned to their normal feeding habits within 8 to 10 hours after surgery. Urination and defecation were normal upto 7th postoperative day. The animals appeared quite alert and responsive. There was no postoperative infection, emphysema, port-site herniation as well as wound dehiscence in any animal.

Clinical Observations

No significant difference in physiological parameters, such as heart rate, respiration rate and rectal temperature, was observed at different time intervals. Heart rate, respiration rate and rectal temperature did not change significantly after surgery, so these variables could not be considered useful in the recognition of postoperative pain.[18] In both the groups, there was a decrease in respiration as well as in heart rate immediately after operation might be attributed to post-anesthetic effect of xylazine, also reported by Ilback and Stalhandske[19] in dogs. Luna et al[20] also reported xylazine and ketamine combination causes reduction in heart rate and respiration rate in dogs.

Hematobiochemical Observations

A significant increase ($p < 0.05$) in neutrophils and comparative lymphopenia was observed on 3rd postoperative day may be the result of release of endogenous glucocorticoids

in response to tissue trauma and inflammation.[21] However, on the contrary, Earley and Crowe[22] reported that surgical castration did not affect any of the hematological parameters from day 0 to 3 after surgery and indicate that the health of the animals was not compromised.

Alkaline phosphatase is a zinc metalloenzymes present in most of the tissues and having very high sensitivity in the dog in comparison to the cat.[23] In the present study, plasma ALP level in animals of both the groups were within the normal limit. But, significant increase ($p < 0.05$) in this enzyme was observed on 3rd postoperative day in both the groups. Increase may be attributed to tissue injury as a result of ischemia reperfusion induced oxidative stress in liver and kidney following capnoperitoneum.[24] It returned to the base level values on 7th day of operation. Acid phosphatase activity derived from lysosomal compartment of cells, predominantly in bone and some extent in other cells, like platelets, erythrocyte and spleen.[25] A significant increase of plasma ACP was observed on 1st and 3rd postoperative day. As there was no significant change between the groups, so the changes in serum ACP levels could not be correlated with the type of tissue injury occurred in both the groups because it is a weak marker of soft tissue injury.

Estimation of Oxidative Stress

A mild, transient increase in LPO was observed in both groups after operation up to 3rd postoperative days. The values returned to base level on 7th postoperative day in all the groups. Bisla et al[26] also reported significant increase in lipid peroxidation values after herniorrhaphy in buffaloes. In both the groups, the catalase activity significantly increased on 1st and 3rd postoperative day. This showed that the production of catalase as an antioxidant enzyme against reactive oxygen species (ROS) enhanced by increased lipid peroxidation. Comparatively, the catalase activity was significantly higher in group II on 1st day postoperatively. Whereas in group I, significant increase was observed immediately after operation. The result of the present study was supported with findings of Kumaraguruparan et al.[27] The increase in lipid peroxidation was accompanied by an enhanced antioxidant status, and also the SOD and CAT provide the first line of defence 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 radical from superoxide radical lowered due to decreased activity of SOD. However, the catalase level was significantly higher in group II indicating more oxidative stress. The increased activity of SOD dismutases the super oxides produced and results in the generation of H_2O_2, which is decomposed by CAT into H_2O and O_2.[28]

Superoxide Dismutase

In the present study, SOD activity was significantly lower in group II upto 3rd postoperative day, but it reached to base values on 5th postoperative day. The SOD, an antioxidant enzyme it catalyzes the conversion of superoxide anion radicals into hydrogen preroxide and oxygen molecule. The decreased SOD after operation attributed to decreased LPO in this group. Fridovich[28] reported that the increased activity of SOD dismutases the superoxides and results in the generation of H_2O_2, which is decomposed by CAT into H_2O and O_2.

Reduced Glutathione

In both the groups a significant increase in the reduced glutathione was observed immediately after operation and on 1st postoperative day. Bisla et al[26] also reported 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 Gouaze et al[29]. Reduced glutathione was required for the disposal of H_2O_2 from erythrocytes by a reaction catalyzed by GPx. This reaction was important because accumulation of H_2O_2 might decrease the lifespan of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin. The present study showed that enhanced production of reduced glutathione occurred in both the groups in order to prevent oxidative stress due to different surgical procedures made on these animals.

Ceruloplasmin

The ceruloplasmin level in plasma remained elevated nonsignificantly up to 7th postoperative day in all the groups. Conner et al[30] also reported increase ceruloplasmin level in plasma after surgical trauma due to acute phase inflammatory process in the dog. Solter et al[31] reported that haptoglobin and ceruloplasmin have greater sensitivity as determinants of inflammation in dogs. Acute phase proteins (APPs) level increases in the circulation after surgery and its associated tissue damage[32,33] and are particularly associated with inflammation.[34]

Hormonal Estimation

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.[35] A significant increase ($p < 0.05$) in plasma cortisol was observed immediately after operation in both the groups. It might be attributed to the effect of

capnoperitoneum. Marcovich et al[36] reported the higher cortisol level at 4 hours interval, whereas the peak cortisol level at 2 hours after laparoscopy in dog has been observed by Hancock et al.[37] Testosterone is a male reproductive hormone often used to evaluate the reproductive status of the animal. Due to the source specificity, testosterone estimation has been indicated to assess the effects of two laparoscopic techniques in the present study. In group I, no significant change ($p > 0.05$) in testosterone was observed at different postoperative time intervals. It might be attributed to bilateral vasectomy which do not exert remarkable effect on steroidogenic functionality of the testicle as also reported by Batista et al.[38] Whereas, in group II, a significant decrease ($p < 0.01$) in plasma testosterone was observed immediately after operation up to 7th postoperative day. It might be due to the reduction of testicular blood flow and loss of testicular interstitial tissue. Pepe et al[39] reported laparoscopic sterilization using on endoscopic stapler application on spermatic cord showed decerase in serum testosterone concentration when postoperative stimulation with hCG in donkeys at 3, 6 and 12 months after surgery. Niu et al[40] reported that following castration, the serum concentration of testosterone decreased rapidly in 2 days. Ortega-Pacheco et al[41] also reported significant decrease ($p < 0.001$) in serum testosterone after surgical castration in dogs.

CONCLUSION

On the basis of the parameters studied, it can be concluded that capnoperitoneum at 10 mm Hg pressure gradient and CO_2 at the flow rate of 2 litre/minute provides optimum visualization of intra-abdominal organs and found suitable for laparoscopic sterilization in male dogs. The laparoscopic vasectomy alone in male dogs was found comparatively quick, less time consuming and can be sucessfully applied for mass sterilization program. Oxidative stress in laparoscopic vasectomy (group I) was less as compared to other group.

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