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Oxidative stress and antioxidant activity in female dogs undergoing laparoscopic and open elective ovariectomy

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ABSTRACT

The present study was carried out to evaluate the biomarkers of oxidative stress and antioxidant activity in female dogs undergoing laparoscopic and open elective ovariectomy at Bihar Veterinary College, Patna in 2016-17. Twenty healthy animals were randomly divided into four groups, viz. A, B, C and D consisting of 5 animals each. The ovariectomy was performed through laparoscopy in Group A and Group B. Open elective ovariectomy procedure was used in Group C and Group D. Evaluation of surgical techniques was done on the basis of biomarkers for oxidative stress and antioxidant activity at Pre, Post and 4th day post surgery. The superoxide dismutase level on day 4th in Group B showed significant difference with highest value followed by Groups A, C and D respectively. The catalase levels on day 4th in Group B were significantly different from Groups A and D but not from Group C. The values within the groups were significantly higher on day 4th as compared to pre and post intervals of time. It can be concluded that less oxidative stress is induced during surgical procedure by laparoscopy as compared to open laparotomy ovariectomy in canines.

Keywords: Canine, Catalase, Molondialdehyde, Oxidative stress, Superoxide dismutase

During aerobic cellular metabolism under physiological conditions, oxygen, the key element required to produce energy for the cellular activity and oxidation of organic compounds, is consumed and reduced, generating a series of highly reactive chemical substances called reactive oxygen species (ROS), also known as free radicals (Dallaqua and Damasceno 2011). Oxidative stress represents an imbalance between the production of ROS and the ability of the antioxidant defence mechanisms to detoxify the reactive intermediates. Certain pathological conditions result in oxidative stress and free radical damage to lipids, proteins, and DNA that has been associated with the initiation, promotion, and progression of carcinogenesis and other chronic diseases (Valko *et al*. 2002, Droge 2007). Commonly measured markers include measures of lipid peroxidation, such as F2a-isoprostanes (isoP) and malondialdehyde (MDA). MDA is currently regarded as a general biomarker of oxidative damage in the plasma (Kadiiska *et al*. 2005). Del Rio *et al.* (2005) inferred that MDA is highly cytotoxic and genotoxic, and should be considered more than just a biomarker of oxidative damage, owing to its interaction with DNA and other proteins. The methods most widely used now-a-days to detect the action of ROS indirectly, include either measuring the products generated from its action on biomolecules or measuring the quality and quantity of antioxidants, thereby evaluating oxidative damage (Mak 2008).

Elective sterilization of female dogs for control of population is one of the most common procedures performed in veterinary practice (Greenfield *et al.* 2004). Many surgical sterilization techniques like traditional midline ovario-hysterectomy (OH), lateral flank OH, castration, ovariectomy and laparoscopic OH are performed (Howe 2006). Laparoscopy is one of the most potent and promising aid for both of its diagnostic and therapeutic use. It requires minimal invasiveness with maximum visibility, shorter surgical time, decreased post-operative discomfort and pain, uncomplicated healing with minimal scarring and minimal surgical morbidity. Currently, the association between oxidative stress and diseases such as cancer, atherosclerosis, fibromyalgia, and autoimmune disorders, in addition to aging-related processes, is well established (Bagis *et al.* 2005). The level of oxidative stress shows degree of cellular damage during surgery that may cause poor outcome in patient and the minimization of oxidative stress is therefore very important. The purpose of our study was to determine biomarkers of oxidative and antioxidative activity in female dogs undergoing laparoscopic and open elective ovariectomy.

MATERIALS AND METHODS

The study was conducted on 20 clinically healthy, adult, female dogs brought by their owners for elective

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spaying during the years 2016 and 2017 in the Department of Veterinary Surgery and Radiology, Bihar Veterinary College, Patna. The whole abdomen of the animal was scanned using ultrasonography for any abnormalities in the reproductive tract like cystic endometrial hyperplasia, pyometra, ovarian tumor, etc. and those animal diagnosed for these abnormalities in the reproductive tract were not included in this study. The study was conducted in two phases (1 and 2). In phase 1, ovariectomy was performed in animals of Groups A and B with laparoscopy whereas, in phase 2, ovariectomy in animals of Groups C and D were done by open laparotomy incision (Table 1). Standard laparoscopic equipments and instruments (M/s Karlstorz., Germany) were used to perform laparoscopic guided ovariectomy. The animals were kept off fed for 12 h and water was withheld for 6 h prior to surgery. The mid-ventral abdomen and right lateral flank skin area were shaved, cleaned, and scrubbed to prepare the surgical site. All animals were pre-medicated with injection Glycopyrrolate at the dose rate of 0.02 mg/kg body weight intramuscularly followed by injection Xylazine HCI at the dose rate of 1.0 mg/kg body weight intramuscularly; induction was done with propofol intravenously and maintained on 2% isoflurane. The animals were placed on dorsal recumbency on V-top table to facilitate cranial displacement of the visceral contents.

Table 1. Experimental design

Group	Number of animals	Design
A	5	Laparoscopic ovariectomy by bipolar electrocoagulation with triangular port
B	5	Laparoscopic ovariectomy by bipolar electrocoagulation with a port in a straight line on the *linea alba*
C	5	Ovariectomy by open laparotomy through the right flank approach
D	5	Ovariectomy by open laparotomy through *linea alba* approach

Surgical procedure: The capnoperitoneum was established by giving a small skin incision of about 0.5 cm caudal to the umbilicus in Groups A and B. Veress needle was inserted at the incision site directing the tip of the needle caudally. Carbon dioxide insufflation at a rate of 2 litres/min to create a pressure gradient (10 to 12 mm of Hg) was made after connecting the CO_2 gas hose from the endoflator. Respiration and capillary perfusion were closely monitored to check overdistension. The Veress needle was removed and a 10 mm safety trocar and cannula were inserted into the peritoneum. The 10 mm telescope was placed through the port to identify the epigastric blood vessels in order to facilitate placement of the two paramedian instrument ports (Left and Right side) 1 cm lateral to the mammary teat on the caudal abdomen under camera supervision (Triangular) in Group A, whereas, it was in a straight line on *linea alba* in Group B (Fig. 1).

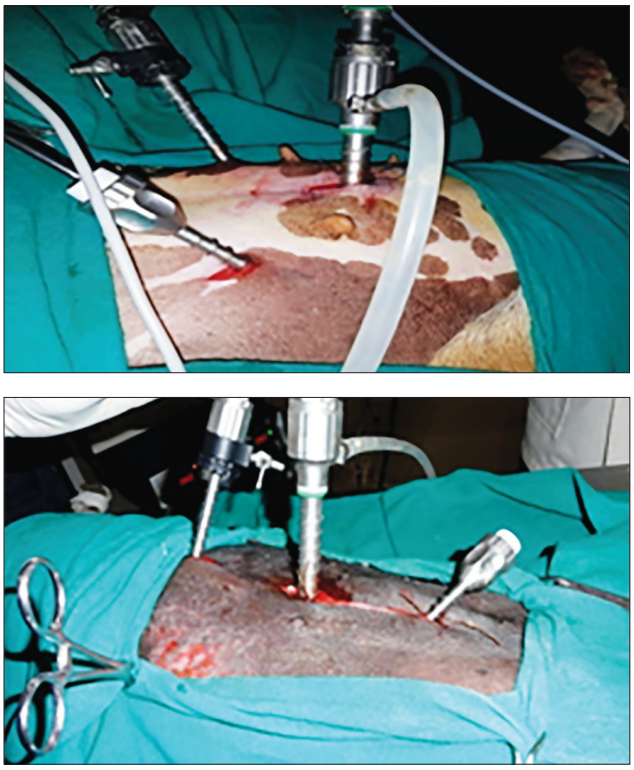

Fig. 1. Placement of port (triangular) in Group A and on *linea alba* (straight) in Group B.

All animals in groups A and B were subjected to laparoscopic ovariectomy with the ovarian vessels cauterized with the help of bipolar electrocautery (Fig. 2). Bipolar laparoscopic forceps were inserted through the left paramedian port and cauterization and coagulation of ovarian blood vessels was done and transection of the ovarian ligament was done to remove the ovary. The procedure was repeated in reverse order for the left ovary. The completely resected ovarian portions were taken outside through a caudal port under the guidance of a 10 mm laparoscope. Each animal of Groups A and B were assiduously inspected in all resected sites for hemorrhage after completion of the procedure.

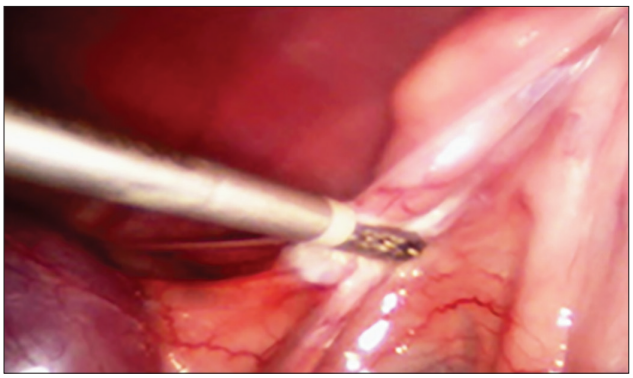

Fig. 2. Electrocoagulation of ovarian vessels with bipolar forceps.

The animals of Groups C and D were subjected to ovariectomy by open laparotomy. The site of incision to approach ovariectomy was the right flank posterior to the

last rib in Group C and ventral midline incision on *linea alba* in Group D (Fig. 3).

After performing laparotomy, the ovarian pedicles were clamped using forceps, double ligated by using 1-0 polyglycolic acid (Vicryl®) and transected. The proper ovarian ligament was then ligated with a suture and the ovaries were removed. In open laparotomy and laparoscopic surgical procedures, the sub-cutaneous tissue and muscle of the ports were opposed with one simple interrupted cruciate suture pattern using 1-0 polyglycolic acid (Vicryl®), followed by skin with a simple interrupted suture pattern with nylon.

Post-surgery wounds of all animals were dressed regularly with povidone-iodine. Broad-spectrum antibiotic Amoxycillin and sulbactam at the dose rate of 10 mg/kg

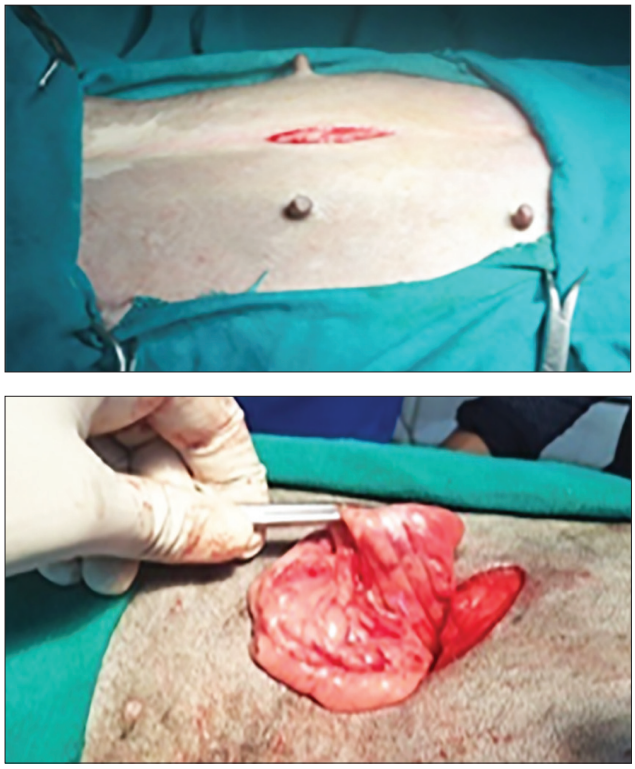

Fig. 3. Skin incision on the right flank in Group C and *linea alba* in Group D for open ovariectomy.

body weight. (Amoxyrum forte®, Virbac Animal Health Care Pvt. Ltd.) was given to all animals for five days and analgesic injection. Meloxicam at the dose rate of 0.2 mg/ kg body weight (Melonex®, Intas Pharma) was given for three days.

Observations (Oxidative stress and antioxidative activity): Plasma samples were used for estimation of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase by a standard protocol at pre, post, and day 4th post-surgery as per standard protocols of Stock and Dormandy (1971), Madesh and Balasubramanian *et al*. (1998) and Aebi (1983), respectively.

Statistical analysis: Analysis of variance (ANOVA) was used to compare the means at different time intervals amongst the groups (Snedecor and Cochran 1994) by using SPSS (2016) Computer package.

RESULTS AND DISCUSSION

Any trauma or injury generates free oxygen radicals in the body which are neutralized by antioxidants like glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and catalase (CAT) thereby, maintaining tissue integrity in living organisms. When antioxidants fail to neutralize oxidants, oxidative stress causes tissue damage due to the increase in toxic products such as malondialdehyde (MDA). Due to the imbalance between pro-oxidant and antioxidant defence, excessive production of reactive oxygen species (ROS-superoxide radical O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) pushes the cell towards oxidative stress (Mukherjee *et al*. 2013).

The present study aimed to report the different approaches for OVE during a laparoscopic and open elective surgical procedure by comparing the oxidative stress in animals undergoing this surgical procedure. As spaying in female dogs is the most common surgical procedure performed in the veterinary practice not only to control the population and to reduce the zoonotic incidence of rabies but also to prevent the risk of development of mammary cancer, pyometra, and elimination of undesirable behaviours associated with hormonal cycling in female dogs. In the present study, OVE has been done as the all cases were screened prior with ultrasonography to rule out any uterine abnormalities with an additional advantage over OVH, it requires a smaller incision, better viewing of the ovarian pedicle, complications such as bleeding, incisional swelling, seroma, infection, dehiscence, delayed healing, self-inflicted trauma and less pain (Van Goethem *et al*. 2006, DeTora and McCarthy 2011).

On pre-treatment days, the Mean±SE values for the MDA among the groups did not show any significant changes whereas, on the post-treatment time, Group B (1.35±0.01) showed significantly lower values than Groups A (1.41±0.01), C (1.45±0.00) and D (1.47±0.00), respectively (Table 2). Though Group C did not show any significant difference from Group D, an albeit lower value in Group C (1.45±0.00) was observed than in Group D (1.47±0.00). On day 4th, Groups A and B were significantly different from Groups C and D. Group C also differed significantly from Group D. Lipids are one of the most susceptible substrate to free radical damage and biomarkers of lipid peroxidation are considered the best indicator of oxidative stress (Georgieva 2005). Malondialdehyde (MDA) is one of the low-molecular-weight end-products formed during the radical-induced decomposition of polyunsaturated fatty acid. The lower values of MDA at different time intervals in Groups B and C might be due to less oxidative stress in animals undergoing laparoscopic surgery with a port placed in a straight line on *linea alba* and open elective surgery through the right flank incision.

In post-treatment, the Mean±SE values of SOD (K/g of protein) in Groups A (177.14±0.71) and

Table 2. Mean±SE of serum lipid (MDA) (nmol malonyldialdehyde/ml of blood serum), superoxide dismutase (SOD) (K/gram of protein) and catalase (CAT) (K/gram of protein) of animals at various time intervals

Parameter	Group	Pre-treatment	Post-treatment	Day 4th (Check)
MDA	A	1.19±0.06^{x}	1.41±0.01^{by}	1.59±0.02^{az}
	B	1.16±0.05^{x}	1.35±0.01^{ay}	1.52±0.01^{az}
	C	1.16±0.05^{x}	1.45±0.00^{cy}	1.69±0.02^{bz}
	D	1.21±0.06^{x}	1.47±0.00^{cy}	1.90±0.03^{cz}
SOD	A	137.94±4.11^{x}	177.14±0.71^{ya}	201.09±1.39^{zb}
	B	141.94±2.95^{x}	181.04±0.35^{ya}	220.65±6.98^{za}
	C	139.54±2.04^{x}	170.40±0.87^{yb}	192.52±1.98^{zbc}
	D	141.56±0.71^{x}	157.41±2.40^{yc}	185.42±0.59^{zc}
CAT	A	2.76±0.08^{x}	3.54±0.01^{ay}	4.05±0.03^{az}
	B	2.81±0.04^{x}	3.62±0.01^{by}	4.37±0.16^{bz}
	C	2.80±0.02^{x}	3.40±0.02^{cy}	3.84±0.04^{bcz}
	D	2.77±0.07^{x}	3.13±0.05^{dy}	3.71±0.01^{cz}

Superscript (a, b, c, d) in column indicates significant difference between the groups within days. Superscript (x, y, z) in row indicates significant difference within group from pre-treatment.

B (181.04±0.35) were significantly higher than in Groups C (170.40±0.87) and D (157.41±2.40) (Table 2). Group C also differed significantly from Group D. Though Group B did not show any significant difference from Group A, albeit higher value in Group B (181.04±0.35) was recorded as compared to Group A (177.14±0.71). On day 4th, Group B showed a significant difference with the highest value followed by Groups A, C and D, respectively. The values within the Groups were significantly higher on day 4th as compared to pre and post-treatment interval. SOD catalyzes the dismutation of superoxide to hydrogen peroxide (H_2O_2) and it is considered the first defence against pro-oxidants. It is a natural antioxidant enzyme against free reactive oxygen radicals; the highest value of SOD in Group B followed by Groups A, C, and D was suggestive of least stress in Group B animals. The least stress in Group B could be due to less formation of free radicals and a less quantity of SOD needed to counter-balance the free radicals and surplus enzymes remain as balance in the system. In dairy goats, SOD activity decreased during the postpartum period probably as a consequence of lower peroxide generation as testified by the decrease in reactive oxygen metabolites (ROM) concentrations (Celi *et al.* 2010).

The Mean±SE values of CAT (k/g of protein) in post-treatment time showed significant difference among the groups with the highest recorded value in Group B (3.62±0.01) followed by Groups A (3.54±0.01), C (3.40±0.02) and D (3.13±0.05), respectively. On day 4th, Group B differed significantly from Groups A, C and D. Although Group C did not differ significantly from Group D albeit higher value in Group C (3.84±0.04) was recorded as compared to Group D (3.71±0.01) (Table 2). The CAT values on post-treatment were also significantly higher within the respective groups as compared to the pre-treatment point of time.

As SOD activity increases H_2O_2 production, protection from reactive oxygen would only be given by a simultaneous increase in catalase and GSH-Px activities and availability of glutathione. Studies in dairy goats have shown that blood GSH-Px activity is decreased during the post-partum period, suggesting that goats may have experienced some degree of oxidative stress and lipid peroxidation (Celi *et al.* 2008, 2010). The time duration from the first incision to the completion of the final knot was considered as a time for surgery. The median time for laparoscopic ovariectomy in Groups A and B was 22 min (16-25 min) and 19 min (15-24 min) respectively, whereas, in the open surgical procedure, it was 26 min (20 to 28 min) in Group C right flank and 28 min (22 to 32 min) in Group D *linea alba*, respectively. Culp *et al*. (2009) reported that the median surgical time of 30 min for laparascopic OVE was significantly longer than traditional ovariohysterectomy (21 min). The less time in the right flank approach might be due to the easy approach to find the location of ovaries just near to the line of incision as compared to *linea alba* incision. It has been reported that once the technique has been mastered, the duration of surgery can be reduced to 15-20 min (Giraldez and Bowlt 2013). The median duration of surgery for laparoscopic OVH as 108 min vs 55 min for the laparoscopic OVE with uneventful recovery has been reported in dogs (Luntang-Jenson 2006).

On the basis of the results of the study, it was concluded that less oxidative stress is induced during a surgical procedure by laparoscopy when ports were placed in a straight line on *linea alba* and right flank site during open laparotomy ovariectomy in canines. This knowledge could contribute both to the basic research and to therapeutic targeting in routine clinical practice. In the future, further surgical stress may be minimized by reducing the number of the ports to minimize surgical trauma in laparoscopic OVE. Local application of slow-releasing analgesics and long-acting antibiotics may further help to minimize the stress in animals requiring multiple post-surgical injections.

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