RESEARCH ARTICLE

Haemato-Biochemical Changes after Laparoscopic and Conventional Ovariohysterectomy in Dogs

Raj Kumar*, Praveen Bishnoi, Mahendra Tanwar, Suresh Kumar Jhirwal, Anita Kumari

Abstract

The present study was conducted on 24 bitches presented for ovariohysterectomy at Veterinary Clinical Complex of the college, Bikaner, India. The animals were randomly divided in two groups. Ovariohysterectomy was performed by conventional method and laparoscopic method in Group A and B, respectively. Physiological and haemato-biochemical parameters were determined in both the groups. The post-operative heart rate, respiratory rate and rectal temperature reduced significantly ($p<0.05$) as compared to pre-operative values in both the groups. PCV values decreased significantly in animals of both the groups. The difference in TEC and TLC values remained non-significant in both the groups; however, in Group-A animals, significant ($p<0.05$) increase in TLC values was noticed at 12 to 24 h. Significant ($p<0.05$) increase in neutrophils and decrease in lymphocytes were recorded, while monocytes and eosinophils remained statistically unchanged during the entire period of time. Significant ($p<0.05$) increase was observed in the values of blood glucose, cortisol, AST, creatinine, CK, alkaline phosphatase in Group-A and Group-B, however, ALT values remained non-significant in both the group. Increased TLC, neutrophils, serum cortisol, AST and CK for longer duration in animals of Group-A compared to Group-B indicated more inflammation, tissue trauma, muscle damage and stressed conditions in conventional method of ovariohysterectomy.

Key words: Biochemical changes, Dog, Haematology, Laparoscopy, Ovariohysterectomy.

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Introduction

The elective sterilization of female canines is typically carried out for population control to prevent reproductive tract disorders, and for the removal of undesired behaviours linked to hormone changes (Kustritz, 2007). For the study of stress response and health status of animals, physiological parameters and haematological biomarkers are frequently examined (Hogland *et al.,* 2014). It is generally known that standard ovariohysterectomy causes tissue trauma, organ manipulation, and inflammation, which result in pain and tension (Lemke *et al*., 2002). In both human and animals cortisol is used for assessing the responses of hypothalamic-pituitary-adrenal axis to environmental changes, anaesthesia and surgery (Church *et al.,* 1994). There are number of physiological reactions to CO_2 pneumoperitoneum or capnoperitoneum that, individually and collectively, have an effect on an animal's cardiopulmonary function, such haemodynamic alterations are brought on by the mechanical and endocrine effects of capnoperitoneum, as well as the effects of absorbed CO_2 on cardiovascular and respiratory function (Windberger *et al*., 1994). Hence the present study was undertaken to evaluate the physiological and haemato-biochemical parameters following conventional and laparoscopic ovariohysterectomy in dogs.

Materials and Methods

The present study was conducted on 24 non-pregnant bitches of varying age and body weight with healthy uterus presented at the Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Science, RAJUVAS, Bikaner (India) for elective sterilization. Apparently healthy dogs were selected for the ovariohysterectomy and all the animals were subjected to a series of diagnostic tests, *viz.*, medical history, clinical symptoms, physical examination, haematology and serum biochemistry. The ultrasonographic examination was also done. Based on the surgical approach the selected bitches were randomly assigned into two groups. In animals of Group-A ovariohysterectomy was performed by conventional method using ventral midline approach, while in Group-B it was performed by laparoscopy guided triple port approach. The animals of both the groups were operated under general anesthesia after premedication with atropine sulphate @ 0.04 mg/kg IM. The anaesthesia

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Table 1: Physiological parameters in bitches of group A and B at different time intervals of ovariohysterectomy (Mean ± SE)

		Preoperative	Post-operative values					
Parameters	**Group**	**30 min**	**30 min**	**6 h**	**12 h**	**24 h**	**48 h**	**Day 10**
Heart rate (Beat/min)	A	110.31 ±0.93d	100.3 ±0.56a	102.5 ±0.57a	105.0 ±0.47b	107.0 ±0.57b	107.5 ±0.68c	109.0 ±0.72c
	B	104.83 ±0.45d	98.66 ±0.35a	100.33 ±0.43b	100.33 ±0.49b	102.83 ±0.36c	104.16 ±0.38d	104.33 ±0.55d
Respiration rate (Breath/min)	A	26.42 ±0.58c	21.25 ±0.46a	20.41 ±0.73a	23.83 ±0.44b	23.91 ±0.52b	26.33 ±0.51c	26.38 ±0.52c
	B	26.41 ±0.58c	21.08 ±0.43a	19.58 ±0.33a	23.5 ±0.41b	24.08 ±0.49b	25.96 ±0.48c	26.10 ±0.51c
Rectal temperature (0F)	A	101.5 ±0.07e	99.64 ±0.12a	100.1 ±0.11b	100.6 ±0.14c	100.7 ±0.15c	101.1 ±0.09d	101.2 ±0.12e
	B	101.27 ±0.12d	99.23 ±0.11a	99.65 ±0.98b	100.27 ±0.13c	100.51 ±0.79c	100.95 ±0.11d	101.33 ±0.85d

Mean ± SEs bearing uncommon superscripts within the row differ significantly ($p<0.05$) from pre operative to post-operative values.

was induced with Xylazine (1 mg/kg b. wt) and Ketamine (10 mg/kg b. wt.) IM and maintained by Isoflurane.

Physiological parameters, *viz.,* heart rate, respiration rate and rectal temperature were recorded during pre and post-operative period at different time intervals. For haematology parameters, blood samples were collected in vials containing EDTA and for biochemical in vials without EDTA at 30 min each pre- and post-operatively followed by on 6th, 12th, 24th, 48th h and 10th day, post-operatively. The biochemical parameters, *viz.,* glucose, cortisol, creatine phosphokinase, ALT, AST, alkaline phosphatase and creatinine were evaluated after separating serum from the blood. The haematological parameters were determined manually and the biochemical parameters were estimated with semi-autoanalyzer (Spectralab Genie, Spectrum Medical Industries Pvt. Ltd.) using standard kits.

The data obtained were statistically analysed and compared as per the standard statistical procedures suggested by Snedecor and Cochran (1994) and significance of mean difference was tested by Duncan's new multiple range test.

Results and Discussion

The data presented in Table 1 reveal that all the physiological indices were almost stabilized and similar to or nearer to the preoperative values by 48 h post-operative period in both the groups. The mean values of heart rate (normal range 70-120/min), respiratory rate (normal range 18-25/min) and rectal temperature (normal range 100.2-103.8°F) were found to be dropped significantly at 30 min post-operative period as compared to preoperative values in both the groups under study. During post-operative period, the values improved gradually and significantly ($p<0.05$) at each interval from 30 min till 48 h (Table 1). Mahalingam *et al.* (2009) reported non-significant decrease in heart rate immediately after the operation in the dogs subjected to laparoscopic sterilization due to post-anaesthetic effect of xylazine. On the contrary, Steinacher and Remedios (1996) recorded significant increase in heart rate from 15 min to 180 min in dogs undergoing laparoscopic surgeries under CO_2 insufflation at 15 mm Hg. In the present study, increased heart rate after 6th h to 10th day may occur as a reimbursing response to decrease venous return after pneumoperitoneum and due to absorption of CO_2 (Fukushima *et al.,* 2011). However, Hancock *et al.* (2005) observed no significant difference in heart rate in dogs after ovariohysterectomy by laparoscopy or median celiotomy.

Respiratory rate decreased significantly ($p<0.05$) in both the groups till 6th h and then resumed preoperative value at 48th h and 10th day. Brzeski *et al.* (2002) and Khandekar (2011) observed decreased respiratory rate after anaesthesia.

A significant decrease in rectal temperature was recorded during anaesthesia at 30th min and 6th h then assumed preoperative values at 12th h onward. Dutta *et al.* (2010) and Hancock *et al.* (2005) observed no significant difference in rectal temperature in dogs after ovariohysterectomy by laparoscopy or median celiotomy.

Haematological Changes

The results of the study in Group-A revealed that the haemoglobin level decreased significantly ($p<0.05$) post-operatively at 30th min, 6th h and 12th h, which returned to preoperative level at 24th h onward, however in Group-B haemoglobin level had no significant change. Results of the present study corroborate with the observations of Rafee *et al.* (2015) who attributed this decrease in haemoglobin in dogs due to shifting of fluid from extravascular to intravascular compartments and haemodilution following fluid therapy during surgery. The PCV values decreased significantly ($p<0.05$) post-operative upto 10th day in both the group. Raibole (2012) also observed similar findings in PCV values when comparing laparoscopic and conventional cryptorchidectomy.

The total leukocyte count was increased significantly at 12 to 24 h in animals of Group-A only, which may be due to infection, tissue trauma or surgical stress. The neutrophils increased significantly (p<0.05) initially in both the groups by 24 h in Group-A and 6 h in Group-B followed by a significant (p<0.05) decrease in both the groups. The lymphocytes decreased significantly from base value upto 6^{th} h post-operatively and then increased significantly in Group-A and non-significantly in Group-B, while the monocytes were decreased at all intervals from 30^{th} min to 10^{th} day post-operatively. Mahalingam *et al.* (2009) observed neutrophilia and compensatory leukopaenia on 3^{rd} post-operative day after performing laparoscopic and open method spaying in bitches. Brzeski *et al.* (2002) observed non-significant increase in neutrophils, lymphocytes and monocytes up to 48 h in dogs during laparoscopic surgeries. The increase in neutrophils was attributed to local inflammation in the wound caused by the trocar introduction. Suresha *et al.* (2012) recorded non-significant neutrophilia at 6^{th} - 48^{th} h post-surgeries in dogs. The mean eosinophils in Group -A reduced non-significantly at 30 min, 6^{th} h, 24^{th} h, 48^{th} h and 10^{th} day post-operatively in comparison to preoperative value, whereas in Group-B initially it increased then decreased significantly. However, all these values were in normal range. Our reports corroborate with Anderson *et al.* (1993) who observed similar findings in eosinophil count post-operatively in dogs.

Table 2: Haematological parameters in bitches of Group A and B at different time intervals of ovariohysterectomy (Mean ± SE)

		Preoperative	Post-operative values					
Parameters	**Group**	**30 min**	**30 min**	**6 h**	**12 h**	**24 h**	**48 h**	**Day 10**
Hb (g/dL)	A	12.70 ±0.20b	11.93 ±0.15a	11.97 ±0.10a	11.99 ±0.23a	12.50 ±0.10b	12.61 ±0.15b	12.68 ±0.50b
	B	12.80±0.71a	12.50 ±0.31a	12.54 ±0.35a	12.59 ±0.28a	12.64 ±0.20a	12.73 ±0.05bc	12.78 ±0.26a
PCV (%)	A	42.11 ±0.71d	38.23 ±0.63c	36.42 ±0.55b	34.9 ±0.67a	35.01 ±0.44a	34.09 ±0.62a	33.81 ±0.68a
	B	41.33 ±0.54 b	39.08 ±0.46a	37.33 ±0.37a	36.92 ±0.55a	38.25 ±0.85a	39.08 ±0.81a	38.42 ±0.66a
TEC (million /µL)	A	6.97 ±0.14ab	6.84 ±0.21ab	6.82 ±0.12ab	6.84 ±0.11ab	6.71 ±0.13ab	6.11 ±0.33ab	5.58 ±0.19a
	B	6.71 ±0.34ab	6.5 ±0.35ab	6.55 ±0.20ab	6.52 ±.034ab	6.38 ±0.68ab	6.36 ±0.27ab	5.88 ±0.24a
TLC ($x10^3/mm^3$)	A	12.45 ±0.24a	13.03 ±0.50a	14.32 ±1.12ab	23.69 ±0.39e	27.69 ±0.23f	22.36 ±0.17d	15.77 ±0.21bc
	B	12.1 ±0.46ab	12.3 ±0.20ab	12.4 ±0.17ab	12.5 ±0.18ab	12.33 ±0.16ab	11.83 ±0.33a	11.39 ±0.11a
Neutrophils (%)	A	67.92 ±0.45a	73.83 ±0.68d	73.67 ±0.51d	75.58 ±0.78d	76.58 ±0.45b	67.5 ±0.37a	67.98 ±0.53a
	B	65.9 ±0.57a	71.00 ±0.66b	72.08 ±0.82c	69.58 ±0.35b	67.25 ±0.41a	66.5 ±0.67a	66.08 ±1.66a
Lymphocytes (%)	A	23.83 ±0.20c	19.75 ±0.35ab	19.17 ±0.34a	20.58 ±0.54b	22.67 ±0.30c	23.67 ±0.39c	22.58 ±0.35c
	B	22.58 ±0.51b	20.01 ±0.55a	19.16 ±0.44a	19.91 ±0.52a	20.09 ±0.50a	22.7 ±0.42b	21.8 ±0.60b
Monocytes(%)	A	4.58 ±0.22c	4.16 ±0.20c	3.01 ±0.17a	2.91 ±0.19a	3.25 ±0.25b	3.15 ±0.10b	3.75 ±0.27c
	B	4.91 ±0.31ab	4.25 ±0.35ab	4.25 ±0.35ab	3.33 ±0.25ab	3.16 ±0.27ab	2.75 ±0.17a	2.91 ±0.25a
Eosinophils (%)	A	3.16 ±0.16a	2.75 ±0.21a	2.41 ±0.14a	2.58 ±0.14a	2.75 ±0.21a	2.75 ±0.17a	3.06 ±0.31a
	B	3.58 ±0.25a	4.16 ±0.29b	3.83 ±0.16b	3.50 ±0.26c	2.83 ±0.24a	2.75 ±0.21a	2.75 ±0.21a

Mean ± SEs bearing uncommon superscripts within the row differ significantly (p<0.05) from pre operative to post-operative values.

Table 3: Biochemical parameters in bitches of Group A and B at different time intervals of ovariohysterectomy (Mean ± SE)

		Preoperative	Post-operative values					
Parameters	**Group**	**30 min**	**30 min**	**6 h**	**12 h**	**24**	**48**	**Day 10**
Glucose (mg/dL)	A	71.5 ± 0.54a	99.25 ±1.30e	90.58 ±0.98d	89.08 ±1.06c	87.92 ±1.92c	76.92 ±0.93b	71.42 ±1.2a
	B	71.17 ±2.57a	93.16 ±1.74e	88.75 ±2.30d	83.58 ±1.24c	80.25 ±1.75c	76.25 ±2.48b	71.83 ±1.82a
Cortisol (µg/dL)	A	2.51 ±0.07 a	5.72 ±0.02g	5.35 ±0.37f	5.05 ±0.11e	4.58 ±0.12d	4.05 ±0.14c	3.06 ±0.06b
	B	2.62 ±0.08a	4.88 ±0.04e	4.61 ±0.09de	4.29 ±0.07c	3.95 ±0.11c	3.52 ±0.12b	2.88 ±0.09a
ALT (IU/L)	A	36.76 ±0.50a	38.18 ±0.28ab	39.15 ±0.70ab	39.23 ±0.30ab	39.60 ±0.16ab	38.18 ±1.14ab	36.5 ±0.51a
	B	31.51 ±0.71a	32.13 ±0.55a	32.34 ±0.56a	32.39 ±0.44a	32.45 ±0.42a	32.44 ±0.56a	30.83 ±0.61a
AST (IU/L)	A	36.57 ±0.18a	38.67 ±0.31b	42.83 ±0.28c	50.98 ±0.37d	54.28 ±0.3e	50.54 ±0.22d	42.49 ±0.46c
	B	27.40 ±0.95a	31.20 ±0.77b	38.33 ±0.24c	41.01 ±0.66d	40.20 ±0.72d	35.38 ±0.93c	30.08 ±0.80a
CK (U/L)	A	206.7 ±1.26a	293.2 ±0.88b	384.3 ±1.36c	664.9 ±1.24g	584.3 ±1.16f	493.0 ±0.90e	458.8 ±1.13d
	B	167.6 ±4.99a	218.7 ±3.45b	362.5 ±5.94e	399.3 ±4.48f	370.5 ±6.13g	329.1 ±7.53d	290.7 ±4.69c
Creatinine (mg/dL)	A	1.05 ±0.016a	1.27 ±0.012b	1.40 ±0.13c	1.45 ±0.09c	1.66 ±0.03e	1.71 ±0.02e	1.533 ±0.02d
	B	1.02 ±0.40a	1.09 ±0.09a	1.26 ±0.02b	1.40 ±0.01b	1.55 ±0.02d	1.45 ±0.21c	1.32 ±0.05b
Alkaline phosphatase (U/L)	A	69.42 ±0.46a	73.67 ±054b	76.5 ±0.43c	90.92 ±0.37d	73.67 ±0.49b	70.83 ±0.29a	74.83 ±0.70b
	B	66.83 ±2.05a	75.92 ±1.33b	77.50 ±0.46b	88.33 ±1.63d	82.25 ±1.00c	69.17 ±0.88a	65.42 ±1.02a

Mean ± SEs bearing uncommon superscripts within the row differ significantly ($p<0.05$) from pre operative to post-operative values.

Biochemical Alterations

In the present study the highest glucose level and cortisol concentration were observed at 30th min post-operative in both the groups followed by gradual declination, which reached to the level of preoperative concentration at 10th day postoperative. Ranganath and Kumar (2007) reported almost three fold increase in blood glucose levels in dogs undertaken to left flank ovariohysterectomy and laparoscopic ovariohysterectomy and attributed the elevated blood glucose levels to stress, pain and increased cortisol levels. Devitt *et al*. (2005) and Hancock *et al*. (2005) reported significant rise in the serum cortisol level in dogs sterilized by open method of ovariohysterectomy in comparison to those sterilized by laparoscopic method. Significant increase in the cortisol level for longer period in Group-A animals compared to Group-B animals suggested that conventional method of ovariohysterectomy is more painful and stressful than laparoscopic method (Devitt *et al*., 2005).

There were no significant changes in ALT activities before and after surgery, whereas a significant increase in AST was found upto 10th day in Group-A, while in Group-B significantly ($p<0.05$) higher AST activity was observed upto 48th h, which decreased at 10th day but was still higher although non-significant as compared to preoperative values. Nan *et al.* (2010), Giraudo *et al.* (2001) and Kumari *et al.* (2018) noticed significant increase in ALT and AST in dogs after laparoscopic surgery. At the same time Rangnath and Kumar (2007) reported significant increase in the AST values in animals that were operated by left flank method of ovariohysterectomy in comparison to those operated by laparoscopic method at 48 h to 72 h post-operatively. Brzeski *et al.* (2002), however, reported no significant change in the activity of ALT in either of the experimental groups. The ALP activity increased significantly ($p<0.05$) upto 12th h, however it remained higher upto 10th day postoperative in both the groups. Our results corroborate with the reports of Mahalingam *et al.* (2009) and Stedile *et al.* (2009), who opined that it may be due to tissue injury as a result of ischemia reperfusion which induced oxidative stress in the liver following capnoperitoneum. Post-operative CK activities increased and highest activity was observed upto 12th h post-operative followed by a declination, however the activity remained higher (Table 3). Our findings corroborate with the reports of Zapryanova *et al*. (2013) and Nan *et al*. (2010). Similarly an increasing trend of creatinine was observed post-operatively. However, Kumari *et al*. (2018) reported non-significant increase in the plasma level of creatinine in post-operative period during ovariectomy via open method and laparoscopic method. Comparatively, more rise in CK and creatinine values in animals of Group-A than Group-B revealed that conventional method caused more tissue trauma and muscle damage as compared to laparoscopic method of ovariohysterectomy (Devitt *et al*., 2005). Significant rise in alkaline phosphate in Group-B was more than Group-A, which denoted caproperitonium condition in laparoscopic method of ovariohysterectomy (Mahalingam *et al.,* 2009).

Conclusions

From the present study, it is concluded that the post-operative changes in haemato-biochemical values indicate that in laparoscopic method of ovariohysterectomy, animals have low inflammation, low pain, low stress and low tissue and muscle damage as compared to animals' undergoing conventional method of ovariohysterectomy.

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