

Effects of Ovariohysterectomy in Dogs and Cats on Adrenocortical, Haematological and Behavioural Parameters

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ABSTRACT

Background: Ovariohysterectomy is considered a prophylactic and therapeutic effect on average better health and reduced mortality rate than intact. Measurement of endocrine, haematological and behavioural responses have been used to evaluate the animal companion response to stressful events. The purpose of this study was to identify the effect of ovariohysterectomy on routinely measures of cortisol response and haematological variables in clinically healthy adult dogs and cats, and to determine whether these parameters could be used to identify a stress response and/or an adaptation to this commonly performed surgery.

Materials, Methods & Results: The study was carried out on 12 clinically healthy female dogs (6 control group and 6 experimental group), Beagle, English Setter, Alsatian breeds, aged 2-4 years, with a mean (sd) weight of 16 (2) kg, and 12 clinically healthy mixed-breed domestic female cats (6 control group and 6 experimental group), aged 2-3 years, with a mean (sd) weight of 4 (1) kg. Sexually intact bitches and cats were randomly assigned to control groups; at the same time, bitches and cats of experimental groups were randomly submitted to ovariohysterectomy, within 2 months after an estrous cycle. Blood samples of experimental groups were taken from a cephalic vein, and only occasionally from a jugular vein in the morning (09:00 a.m.), immediately before ovariohysterectomy and one hour after. Baseline blood samples of control groups were taken in the morning (09:00 a.m.). Owners were interviewed via questionnaire at the time the female dogs and cats were spayed and 2 months later. Dogs and cats showed only slightly decrease of cortisol concentrations after surgery compared to pre-ovariohysterectomy values. In addition, baseline cortisol values of control dog and cat groups were superimposable to pre-ovariohysterectomy values. Related to haematological variables no significant differences between pre-ovariohysterectomy and baseline values were observed in both species. No significant behavioural changes were observed within 2 months after surgical sterilization. Cortisol concentrations, haematological variables and behaviour are not significantly affected by ovariohysterectomy in clinical healthy adult companion animals.

Discussion: The results obtained are in line with what it would have been extrapolated from laboratory studies on the effects of gonadectomy on physical, physiological and behavioural problems. The reason why we did not observe any significant differences of cortisol concentrations between control and experimental groups in our study might be that pure-breed dogs and middle-breed cats used were very homogenous for gender (only females), age (adult subjects), clinically healthy status, and body weight. No significant changes of haematological variables between pre- and post-ovariohysterectomy were supported by successful ovariohysterectomized animals, without complications. Moreover, increases of WBC and PLT count after ovariohysterectomy, in bitches and cats, confirmed data observed in male cats after castration under general anaesthesia with or without local anaesthesia. From the findings obtained in this study we can conclude that cortisol concentrations and haematological variables, body weight and behaviour are not significantly affected by ovariohysterectomy in clinical healthy adult companion animals.

Keywords: cortisol, haematological variables, ovariohysterectomy, dog, cat.

INTRODUCTION

Gonadectomy in females included both ovariectomy and ovariohysterectomy surgical methods, and recent review showed the related short- and long-term risks and benefits of gonadectomy of dogs [7,22] and cats [1,10]. Hence, according to genetic predisposition, gonadal hormones appear to mainly influence the pathogenesis of mammary tumors [20]. On this basis ovariohysterectomy is considered a prophylactic and therapeutic effect on average better health and reduced mortality rate than intact, and it is still recommended to avoid high population densities, animal suffering and the spread of zoonoses [22].

Ovariectomy or ovariohysterectomy usually results in complete and immediate elimination of female sexual behaviour, but there are controversial questions about changes in non-sexual behaviour associated with the operation [8,27]. Increased synthesis of catecholamines and cortisol after anaesthesia, surgery and post-operative pain are part of the neurohumoral stress response and, despite its limitations, cortisol is widely used as an indicator of stress [14,21,25]. Measurement of endocrine, haematological and behavioural responses have been used to evaluate the animal companion response to stressful events and to evaluate the effectiveness of analgesics following painful procedures [2,5,9,26]. The purpose of this study was to identify the effect of ovariohysterectomy on routinely measures of adrenocortical response, haematological variables and behavioural changes in clinically healthy adult dogs and cats, and to determine whether these parameters could be used to identify a stress response and/or adaptation to this surgery methods.

MATERIALS AND METHODS

Animals

The study was carried out on 12 clinically healthy female dogs (6 control group and 6 experimental group), Beagle, English Setter, Alsatian breeds, aged 2-4 years, with a mean (sd) weight of 16 (2) kg, and 12 clinically healthy mixed-breed domestic female cats (6 control group and 6 experimental group), aged 2-3 years, with a mean (sd) weight of 4 (1) kg. Animals were evaluated according to the history, the absence of any previous illness and the absence of drug or dietary supplement. General clinical examinations were performed with recording details. All animal were submitted to conventional vaccination and deworming protocols.

Sexually intact bitches and cats were randomly assigned to control groups; at the same time, bitches and cats of experimental groups were randomly submitted to ovariohysterectomy, within 2 months after an estrous cycle; this preference was based on the assumption that future uterine pathology could be prevented by removing the uterus.

Surgical procedures

To ensure maintenance of an adequate plane of anaesthesia, individual patients were carefully monitored by trained and educated observers. Combining multiple analgesic agents in a single protocol was known as multimodal analgesia, and greatly improves pain and stress control in animals undergoing neutering through a spay-neuter surgery. Bitches were sedated with acepromazine maleate 0.5 mg kg⁻¹ IM (Killitam®)¹, zolazepam 0.7 mg kg⁻¹ IM (Zoletil®)², dexmedetomidine hydrochloride 0.08 mg kg⁻¹ IM (Dexdomitor®)³ and Tramadol clorhidrato 2 mg kg⁻¹ IM (Contramal®)⁴.

Cats were sedated with medetomidine hydrochloride 5 mg kg⁻¹ IM (Dormitor®)⁴, fentanyl citrate 4 mg kg⁻¹ EV (Fentanyl®)⁵ and propofol 3 mg kg⁻¹ EV (Propovet®)⁶ for analgesia, and supplemented with isoflurane-anaesthesia following premedication.

During surgical procedure, all subjects were submitted to cefazoline 30 mg kg⁻¹ EV (Cefazolina®)⁷ and Ringer lactate 10 mg kg⁻¹/h EV (Ringer lattato®)⁸ and after with tramadol 3 mg kg⁻¹ IM (Contramal®)⁴.

Cardiovascular, respiratory and central nervous system functions were continuously monitored so that anaesthetic depth could be modified as needed.

On the experimental groups ventral midline routine ovariohysterectomy were performed. Onset time of analgesia was 4.5 min and duration of analgesia was about 90 min. Duration of surgery was calculated as the time interval between skin incision and skin suturing (within 50 min).

Hormone and haematological analysis

Blood samples of experimental groups were taken from a cephalic vein, and only occasionally from a jugular vein in the morning (09:00 a.m.), immediately before ovariohysterectomy and one hour after. Baseline blood samples of control groups were taken in the morning (09:00 a.m.). Owners were interviewed via questionnaire at the time the female dogs and cats were spayed and 2 months later. Owners of a control groups

of unsprayed dogs and cats were also interviewed twice at an interval of 1 month. In both groups there were changes over time in some dogs and cats but the only two behavioural patterns in which spayed females showed a change that no differed significantly from the control group, and it was in restlessness behaviour towards their own species or towards people after short road transportation in dogs, and in capricious eating in cats. Postoperative evaluations included assessments for normal function, ambulation and respiratory rate and character and for adequate analgesia. Serum total cortisol concentrations were analysed in duplicate through a competitive enzyme assay supplied by (SEAC/RADIM)⁹. During the first incubation, sample cortisol competed with the cortisol conjugated to horse-radish peroxidase (HRPO) for binding to the specific sites of the antiserum coated on the wells. Following incubation, all unbound material was removed by aspiration and washing. The enzyme activity, which was bound to the solid phase, was inversely proportional to the cortisol concentration in calibrators and samples, which was evidenced by incubating the wells with a chromogen solution (tetramethylbenzidine, TMB) in a substrate-buffer. Colourimetric reading was carried out using a spectrophotometer at 450 and 405 nm (Sirio S)⁹. The assay sensitivity was 5 ng/mL. The intra- and inter-assay coefficients of variation (CVs) were 5.5% and 6.8%, respectively.

Statistical analysis

Data are presented as mean values \pm standard deviation (sd) of duplicate measurements. To compare post- with pre-ovariohysterectomy values, a paired *t*-test was applied, respectively for dogs and cats. To compare control groups with experimental groups, a paired *t*-test was applied. The level of significance was set at $P < 0.05$.

All calculations were performed using the PRISM package (GraphPad Software Inc, San Diego, California, USA).

RESULTS

Dogs

Bitches were successfully ovariohysterectomized without complications. Convalescence period was short (1 ± 0.5 days) and owners' satisfaction high. Circulating cortisol concentrations (Figures 1 & 2) and different haematological variables (Tables 1 & 2) of dogs

of each group were in the normal range [15-17], and no significant changes were seen throughout the control and experimental groups. However, cortisol concentrations of cats were higher than those reported in literature [14].

Dogs showed a moderate decrease of cortisol concentrations after surgery (94.31 ± 22.55 nmol/L) compared to pre-ovariohysterectomy (107.97 ± 17.15 nmol/L) values. In addition, baseline cortisol values of control group (106.10 ± 24.60 nmol/L) were similar to pre-ovariohysterectomy values.

Related to haematological variables, red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) after surgery were lower than pre-ovariohysterectomy and baseline values. Higher white blood cell (WBC) and platelet (PLT) counts were observed after ovariohysterectomy than pre-ovariohysterectomy and baseline counts.

Cats

Cats were successfully ovariohysterectomized without complications. Convalescence period was short (1 ± 0.5 days) and owners' satisfaction high.

Cats showed only slightly decrease of cortisol concentrations after surgery (98.51 ± 26.13 nmol/L) compared to pre-ovariohysterectomy (105.31 ± 22.24 nmol/L) values. In addition, baseline cortisol values of control group (101.65 ± 19.23 nmol/L) were similar to pre-ovariohysterectomy values.

Related to haematological variables, RBC, Hb, PCV, MCV, MCH and MCHC values after surgery were lower than pre-ovariohysterectomy and baseline values. Higher WBC and PLT counts were observed after ovariohysterectomy than pre-ovariohysterectomy and baseline counts.

During the first 2 months after ovariohysterectomy, no differences were observed between the control and experimental groups, both for bitches and cats, in terms of general activity, barking, playfulness and affection to wards people. Moreover, two bitches of experimental group displayed restlessness behaviour towards their own species or towards people after short road transportation.

In addition, three cats of experimental group showed only a capricious appetite during the first 15 days after surgery.

However, no significant body weight increases was observed in both bitches and cats.

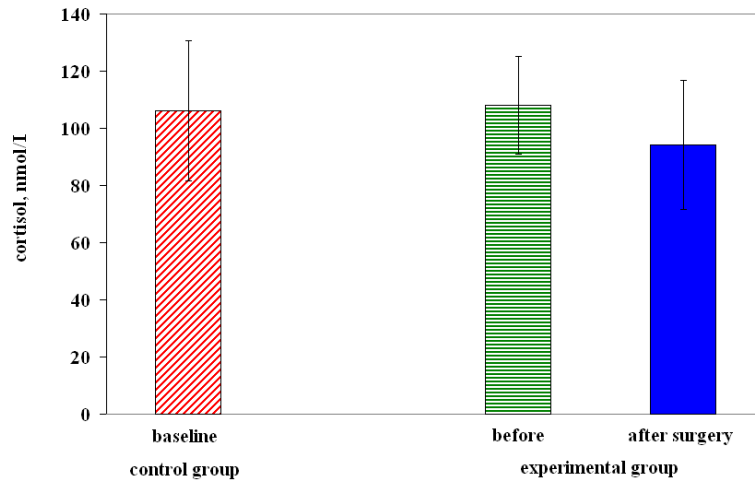


Figure 1. Mean circulating cortisol concentrations (mean \pm sd) of intact bitches (control group) and ovariohysterectomized bitches (experimental group).

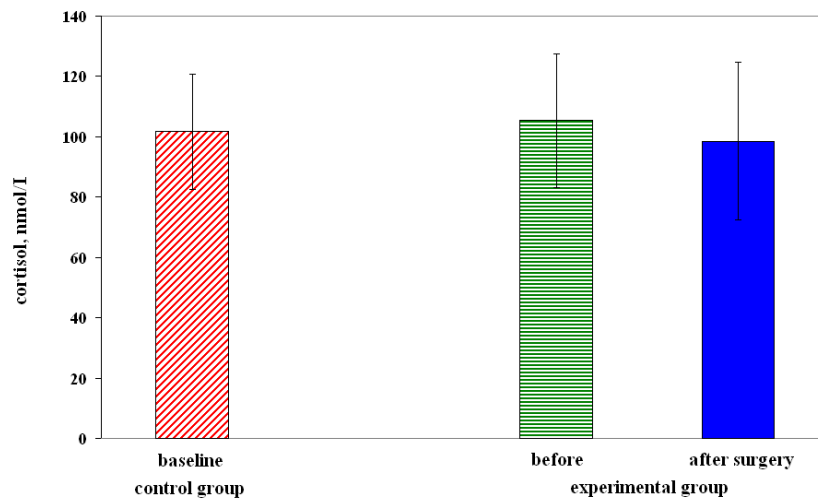


Figure 1. Mean circulating cortisol concentrations (mean \pm sd) of intact cats (control group) and ovariohysterectomized cats (experimental group).

Table 1. Haematological variables (mean \pm sd) of intact bitches (control group) and ovariohysterectomized bitches (experimental group).

Variable	Control group	Experimental group	
	baseline	before surgery	after surgery
RBC (M/ μ L)	7.05 \pm 0.16	7.56 \pm 0.36	6.85 \pm 1.16
Hb (g/dL)	12.05 \pm 1.16	13.00 \pm 0.26	12.05 \pm 0.56
PCV (%)	49.05 \pm 3.16	51.00 \pm 2.86	44.75 \pm 2.56
MCV (fL)	63.05 \pm 2.16	66.00 \pm 1.86	64.75 \pm 2.56
MCH (Pg)	24.01 \pm 1.16	25.55 \pm 0.86	24.85 \pm 0.56
MCHC (g/dL)	33.50 \pm 2.56	33.60 \pm 0.56	31.85 \pm 1.16
WBC (K// μ L)	10.05 \pm 1.16	10.56 \pm 0.56	13.09 \pm 2.16
PLT (K// μ L)	147.05 \pm 30.16	172.50 \pm 28.35	192.50 \pm 32.05

Table 2. Haematological variables (mean \pm sd) of intact cats (control group) and ovariohysterectomized cats (experimental group).

Variable	Control group baseline	Experimental group	
		before surgery	after surgery
RBC (M/ μ L)	8.05 \pm 0.46	9.36 \pm 1.33	8.25 \pm 1.16
Hb (g/dL)	10.15 \pm 1.17	10.80 \pm 0.28	10.05 \pm 0.56
PCV (%)	32.85 \pm 1.16	32.10 \pm 1.86	31.65 \pm 2.46
MCV (fL)	43.00 \pm 1.16	44.00 \pm 1.46	43.44 \pm 2.06
MCH (Pg)	13.76 \pm 1.18	14.35 \pm 0.76	13.30 \pm 0.26
MCHC (g/dL)	31.20 \pm 2.06	33.00 \pm 1.56	31.85 \pm 1.17
WBC (K/ μ L)	12.05 \pm 2.14	12.86 \pm 1.56	13.95 \pm 2.06
PLT (K/ μ L)	177.00 \pm 33.16	182.50 \pm 32.05	202.00 \pm 36.05

DISCUSSION

The results obtained are in line with what it would have been extrapolated from laboratory studies on the effects of gonadectomy on physical, physiological and behavioural problems [7,10]. Although clients sometimes request sterilization without gonadectomy, so that their animals may continue to experience normal reproductive behaviour, the high incidence of mammary neoplasia, pyometra and other diseases in companion animals suggest that bilateral gonadectomy is preferable. Moreover, the optimal age at which to neuter a dog or cat and minimize surgical complications and undesirable effects is not known [12,27], data obtained showed that clinically healthy adult bitches and cats represent no at risk patients. Unfortunately, scientific knowledge related to the ideal age for the elective surgery does not exist [11,24,29]. Furthermore, some differences often are limited by a wide age range of animals in different physical and physiological conditions. The reason why we did not observe any significant differences of cortisol concentrations between control and experimental groups in our study might be that pure-breed dogs and middle-breed cats used were very homogenous for gender (only females), age (adult subjects), clinically healthy status, and body weight; thought, both dogs and cats were fed with a fixed amount of commercial balanced animal food, once a day; in addition, the study was ended within 2 months after an estrous cycle of companion animals used, so all animals were at the same physiological reproductive state. Administering a single injection that includes anxiolytic, analgesic and anaesthetic

induction agents substantially reduced patient pain and stress; thus, combining premedications and anaesthetic induction agents in a single injection was advised, as confirmed by the lowest cortisol concentrations observed after surgery, both in bitches and cats. Cortisol decreases observed in post-ovariohysterectomized companion animals did not confirmed elevated plasma cortisol concentrations found at hours 3 and 6 in surgery dogs, whereas the plasma cortisol concentration in the control groups did not change [5]. On the other hand, data obtained confirmed previous data observed in dogs after ovariohysterectomy that showed a reduction of plasma cortisol concentration in the surgery with oxymorphone group compared to the surgery with placebo (Saline solution) group at hours 3-12, suggesting an analgesic effect of the drug [5]. Additionally, data obtained confirmed both changes in plasma cortisol concentrations obtained in bitches in response to different combinations of halothane and butorphanol, with or without ovariohysterectomy [3], and that various analgesic techniques suppressed surgical stress response after ovariohysterectomy [4,9,15]. Previous studies have shown satisfactory agreement between pain score and plasma catecholamine and serum cortisol levels [16,17,30]. In the current study tramadol was effective at preventing the postoperative stress response, using circulating cortisol as a guideline, confirming data reported for postoperative analgesia in female dogs undergoing ovariohysterectomy [19]. With regard the behavioural findings in the companion manuscript [6] there were disparities between previously reported findings, that suggested that ovariohysterectomy is a stressful experience for

the dog and is associated with marked behavioural changes for a least 24 h after surgery, and the results of our study. In addition, although a cortisol increase was observed in dogs [5] and cats [26] after stress response to this commonly performed surgery, the decreased cortisol concentrations observed after ovariohysterectomy could be due to limited duration of surgical sterilization. No significant changes of haematological variables between pre- and post-ovariohysterectomy were observed; these data were supported by successful ovariohysterectomized animals, without complications. Moreover, increases of WBC and PLT count after ovariohysterectomy, in bitches and cats, confirmed data observed in male cats after castration under general anaesthesia with or without local anaesthesia. No significant body weight increases were observed both in bitches and cats within 2 months later. This may be due to feeding the companion animals that were submitted to a regulated amount of pet food according to the manufacture's recommendation instead of *ad libitum* feeding, since *ad libitum* feeding after ovariohysterectomy was found to result in significant weight gain whereas feeding with a fixed amount of commercial pet food did not result in obesity [10,28,29]. From the findings obtained in this study we can conclude that cortisol concentrations and haematological variables, body weight and behaviour are not significantly affected by ovariohysterectomy in clinical healthy adult companion animals. Administering a single injection that includes anxiolytic, analgesic and anaesthetic induction agents could be substantially reduced patient pain and consequently stress. These observations supported the hypothesis that the limited duration of surgical sterilization performed would reduce the cortisol response after surgery under monitored level of

sedation and appropriate analgesia. That the pre- and post-operative cortisol values were within the dog's reference range suggests that pre- and post-operative stress was minimal; conversely, in cats, factors such as fear and anxiety probably caused the highest endocrine response in catabolic cortisol effects, such as showed by higher cortisol values than physiological range already in pre-operative experimental group and control values. Hence, the measurement of circulating cortisol concentrations best reflects the stress response at the time of blood sample collection, especially for cats. The increased secretion of glucocorticoids in response to stress is immediate, thus, the concentration of cortisol increases within minutes in the bloodstream and confirm its expression of severity of stress.

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Ethical approval. This study was performed according to the guideline of the Italian Ministry of Health, and was formally approved by Ethological Committee of the Department of Veterinary Sciences.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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