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Evaluation of antioxidant enzymes and lipid peroxidation before and after ovariohysterectomy in queen

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Abstract

Estrogens are known to have antioxidant properties and their deficiency after ovariohysterectomy (OVH) predisposes the body to increased reactive oxygen species (ROS) production. Occurrence of oxidative stress following OVH in dogs and rats has been demonstrated in previous studies. However, no investigations have been done in relation to changes in the activity of antioxidant enzymes and lipid peroxidation, especially in cats. The aim of this study was to evaluate the activity of some antioxidant enzymes, malondialdehyde (MDA) concentration as an indicator of lipid peroxidation and 17-beta estradiol in serum before and one month after OVH. In this study, 12 cats aged 2 to 5 years that were not in the estrous cycle were used. Then OVH surgery was performed. Blood samples were taken before and one month after surgery and the activity of superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase (CAT) enzymes, the concentration of MDA and 17-beta estradiol in serum were measured. The activity of SOD, Gpx, CAT and MDA concentration increased after OVH compared to before which was not significant. The concentration of 17-beta estradiol decreased postoperatively compared to before, which was statistically significant. OVH did not cause oxidative stress with no significant change in antioxidant enzymes and lipid peroxidation in cats after one month, although 17-beta estradiol showed a significant decrease. More studies are needed to determine further dimensions.

Key words: Ovariohysterectomy, Antioxidant enzymes, Lipid peroxidation, queen, Oxidative stress

Introduction

Selective spaying of female dogs and cats is one of the most common surgeries performed in veterinary medicine. OVH in small animal veterinary surgery involves the removal of sex hormone-producing organs and the uterus. The most important reasons for doing this include preventing unwanted pregnancy, reducing the risk of development of mammary tumor, pyometra, eliminating estrus and related problems, and preventing an increase in the cat population (Sakundech *et al*.,2020).

Studies in humans and on laboratory animals have shown that a decrease in serum estrogen concentration after ovariectomy is responsible for a change in the antioxidative/oxidative balance, which leads to oxidative stress (Szczubial *et al.*, 2015). It is known that an altered oxidant-antioxidant balance is a risk factor for the development of various pathological states, such as cardiovascular diseases, renal diseases, osteoporosis and Parkinsonism in women who have undergone bilateral

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ovariectomy (Silbiger and Neugarten, 2003; Shuster *et al.,* 2008; Gutierrez-Grobe, *et al.*, 2010). Also, in spayed female dogs, the risk of the development of some diseases, such as some tumors, hip dysplasia and urinary incontinence is increased (Root Kustritz, 2012).

OVH is the most common method of contraception in pets. Many cats are exposed to spaying every year. However, there is lack of information in the literature concerning the oxidative stress and the role of 17-beta-estradiol as an antioxidant hormone in ovariohysterectomized cats.

Oxidative stress is caused by an imbalance between the production of free radicals and reactive oxygen species on one hand and the antioxidant defense system on the other. Some components of this defense system, such as superoxide dismutase, glutathione peroxidase, catalase, and thiol-containing molecules, are produced in the body, but others, such as vitamin E, vitamin C, and beta-carotene, must be obtained through diet. In the of oxidative stress condition, many macromolecules are damaged and the lipid peroxidation process, DNA and protein oxidation, enzyme inactivation, and disorder of animal physiological and metabolic processes occur (Birben *et al.*, 2012; Buege & Aust, 1976; Trevisan *et al.*, 2001).

Estrogens are known to have antioxidant properties and their deficiency after OVH predisposes the body to increased reactive oxygen species (ROS) production. Estradiol sequentially activates MAP kinase (MAPK) and nuclear factor kappa B (NFkappaB) following receptor activation to up-regulate the expression of antioxidant enzymes, providing a cogent explanation for the antioxidant properties of estrogen and its effects on longevity-related genes (Borrás *et al*,2005).

17-beta estradiol (17β-E2), constitute the main product of ovarian granulose cells. 17β-E2 participates in the oxidative balance by preventing the production of ROS in mitochondria. The antioxidant and ROS eliminating activity of 17β-E2 is associated with its chemical structure which has an intact OH group in the phenolic A ring of the molecule (pech *et al,* 2019).

However, no investigations have been done in relation to changes in the activity of antioxidant enzymes and lipid peroxidation after OVH, especially in cats. The aim of this study was to investigate the serum changes of some antioxidant enzymes and lipid peroxidation of female cats one month after surgery.

Materials and Methods

In this study, 12 female cats aged between 2 and 5 years old were used. These animals underwent complete vaccination and antiparasitic treatment, and were not in the sexual cycle.

Ovariohysterectomy

Cats were sedated with the combination of xylazine 1mg/kg (Alfasan Co.) and, acepromazine 0.1 mg/kg (Alfasan Co.), administered intramuscularly. Then, anesthesia was induced using a combination of ketamine 6 mg/kg (Alfasan Co.) and diazepam 0.2 mg/kg (Caspian Co.). After preparing the animal under aseptic conditions, OVH was performed according to the usual methods (Pereira *et al.,* 2018).

Blood sample

Blood samples were taken from the cephalic vein one hour before ovariohysterectomy and one month later. Blood samples were centrifuged in anticoagulant tubes at 3000 rpm for 10 minutes. The serum of each sample was transferred into 0.5 ml vials, and stored in a -70 ° C until the experiment was performed.

Antioxidant enzymes activities

Glutathione peroxidase

Glutathione peroxidase activity was evaluated by Paglia and Valentine's method (1967), using (RANSELKit, Randox, UK). Glutathione peroxidase catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of

glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was measured.

Superoxide dismutase

Superoxide dismutase activity was measured by an iodophenyl nitrophenol phenyltetrazolium chloride modified method (RANSOD Kit, Randox, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4- iodophenyl)-3-(4-nitrophenol)-5-phenyl-tetrazolium chloride (INT) to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction. One SOD unit was considered as that which caused a 50% inhibition of the reduction rate of INT under the assay condition (McCord & Fridovich, 1969).

Catalase

Catalase activity was measured according to Aebi (1984). Decomposition of H2O2 was followed directly by monitoring the decrease of absorbance at 240 nm. Enzyme activity was calculated as catalytic content of a sample.

Determination of MDA concentration

Measurement of serum MDA was performed in serum by thiobarbituric acid method, for which reactive substances were measured with thiobarbituric acid as peroxidation index. The basis of this measurement is the MDA reaction with thiobarbituric acid in the serum, which results in the formation of pink color, and is calculated by measuring the intensity of the color produced at 520 nm (Placer *et al.*, 1966).

The concentration of 17-beta-estradiol in serum samples were measured with ELISA kits (Monobind Inc Lake Forest, CA, USA) according to the manufacturers' protocol using ELISA microplate reader (ELx808, Biotek Instruments, Winooski, VT, USA).

Statistical analysis

The Paired sample t-test was used for comparisons between the pre- and post-OVH cats. All statistical analyses were performed using SPSS 24 statistical program. All data were expressed as mean ± SE. $P<0.05$ was considered as statistically significant.

Results

Antioxidant enzyme activity and 17-beta estradiol

Comparison of mean and standard error of antioxidant enzyme activity and 17-beta-estradiol concentration is shown in Table 1. The mean activity of SOD, Gpx and CAT after OVH compared to before OVH indicated an increase, although this difference was not statistically significant ($p\leq0.05$). Comparison between the two groups demonstrated that the concentration of 17-beta estradiol after OVH was a significant decrease in comparison to before OVH ($p\leq0.05$).

Table1: Comparison of mean ± SE of antioxidant enzymes and 17-beta estradiol, before and after OVH

Parameter	Before OVH	After OVH
SOD(u/ml)	458.75±50.37	546.45±55.55
Gpx(u/ml)	212.40±21.42	254.40±17.40
CAT(u/l)	168.85±12.82	187.65±10.18
17-beta-estradiol(pg/ml)	46.29±8.19	33.29±4.48*

*****: indicates a significant difference between the groups.

Evaluation of lipid peroxidation

Comparison of mean and standard error of malondialdehyde concentration as an indicator for evaluating lipid peroxidation is revealed in Table 2. Comparing the mean MDA between the two groups found that

the concentration of malondialdehyde after OVH increased compared to before OVH but was not significant ($p \leq 0.05$).

Table 2: Comparison of mean ± SE of malondialdehyde, before and after OVH

Parameter	Before OVH	After OVH
MDA(µmol/l)	27.27±1.36	30.69±1.8

Discussion

Estrogens are known to have antioxidant properties and their deficiency after ovariohysterectomy (OVH) predisposes the body to increased reactive oxygen species (ROS) production. 17- Beta- estradiol can preclude oxidation by the hydroxyl group in its phenolic ring. Removal of the uterus and ovaries leads to the elimination of sex hormones. This study was performed to evaluate the serum changes of some antioxidant enzymes and lipid peroxidation in female cats before and one month after ovariohysterectomy.

Evaluation of antioxidant enzyme activity and 17-beta-estradiol concentration

The activity of superoxide dismutase, catalase and glutathione peroxidase increased one month after ovariohysterectomy compared to before surgery, although increase in the activity of these enzymes was not significant. The occurrence of oxidative stress following ovariohysterectomy has been demonstrated in many previous studies (Gunay el al., 2011; Pech et al., 2019; Szczubial et al.,2015). After oxidative stress, superoxide radicals are produced. If these active radicals are not removed, there is possibility of macromolecules damage, lipid peroxidation, and oxidation of DNA and proteins. SOD is a primary line of defense agents against oxygen derived free radicals, and converts them to peroxide hydrogen which is less harmful than the superoxide radical (Sindhu et al.,2004). Following the increase of SOD activity and the accumulation of hydrogen peroxide, other antioxidant enzymes, including GPx and CAT, became involved. The affinity of GPx for H_2O_2 was stronger than that of CAT, which makes it more efficient at low levels of H_2O_2 (Kalpakcioglu & Senel, 2008). Catalase plays a key role in the removal of H_2O_2, and there was a linear correlation between catalase activity and hydrogen peroxide concentration (Mueller et al., 1997). 17-Beta-estradiol has an antioxidant property due to its hydroxyl group on its phenolic ring. Concentrations of this hormone in spayed cats are anticipated to decrease after ovariaohysterectomy, which is one of the reasons for the occurrence of oxidative stress in these animals. In the present study, as expected, the mean concentration of 17-beta-estradiol decrease significantly one month after ovariaohysterectomy compared to before surgery. A slight increase in the activity of antioxidant enzymes was to compensate for the decrease in 17-beta estradiol to prevent oxidative stress, damage to macromolecules, and lipid peroxidation due to the production and accumulation of free radicals. It is better to measure the activity of enzymes and the concentration of malondialdehyde in a longer period of time (2 months, 3 months and 6 months) after OVH to observe the trend of changes accurately and significantly.

Sakundech et al. (2020), Reported that 14 days after ovariohysterectomy in dogs, total antioxidant capacity and catalase activity increased compared to before surgery. Studied the effects of ovariohysterectomy on oxidative stress in female dogs, showed that the activity of Gpx and SOD enzymes increased significantly on day 14 after surgery and then decreased until day 30 after surgery (Szczubial et al., 2015). Evaluation of the effects of ovariaohysterectomy on antioxidant enzymes in female dogs, demonstrated that the activity of Gpx and SOD enzymes did not change one month after surgery compared to before it. Six months after surgery Gpx and SOD activity showed an increase that was statistically significant and 17-beta estradiol decreased significantly (Pech et al., (2019).

Anadol et al. (2016), investigated the effect of ovariohysterectomy in mice, and indicated that there was no significant difference in Gpx and SOD activity on day 10 after surgery compared to day 0 (before surgery). In the other study Gomez et al. (2020) revealed that SOD activity increased in rats 15 days after OVH. Azevedo et al. (2001), observed that Gpx and SOD activity did not change in rats 30 days after ovariectomy.

Evaluation of malondialdehyde concentration

Malondialdehyde is one of the indicators of lipid peroxidation and its increase demonstrated the occurrence of oxidative damage of lipids. The results of this study showed that the concentration of malondialdehyde after OVH increased compared to before OVH, although this increase was not statistically significant.

Lipid peroxidation index, including malondialdehyde, increases significantly in dogs 24 hours after ovariohysterectomy, which is due to surgical oxidative stress (Gunay et al., 2011; Gunes et al., 2008).

Szczubial et al. (2015) evaluated the effect of ovariohysterectomy on oxidative stress in female dogs, and revealed that MDA levels increased on days 14 and 30 after surgery.

Evaluation the influence of duration on pain stress, oxidative stress and total antioxidant capacity in female dogs undergoing ovariohysterectomy showed that malondialdehyde levels increased 3 days after surgery and then decreased until day 14 (Sakundech et al., 2020).

Anadol et al. (2016) studied the effect of ovariohysterectomy in the rats, and showed that MDA consentration increased on day 10 after surgery compared to day 0 (before surgery).

In Conclusion OVH did not cause oxidative stress with no change in antioxidant enzymes and lipid peroxidation in cats after one month, although 17-beta estradiol showed a significant decrease. More studies are needed to determine further dimensions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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بررسی آنزیم‌های آنتی‌اکسیدانی و پراکسیداسیون لیپیدی قبل و پس از اواریوهیسترکتومی در گربه‌های ماده

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چکیده

استروژن‌ها دارای خواص آنتی‌اکسیدانی هستند و کمبود آن‌ها پس ازاواریوهیسترکتومی (OVH) بدن را مستعد افزایش تولید گونه‌های اکسیژن فعال (ROS) می‌کند. وقوع استرس اکسیداتیو به دنبال OVH در مطالعات قبلی در سگ‌ها و موش‌ها به اثبات رسیده است. با این حال تا کنون، هیچ تحقیقی در رابطه با تغییرات در فعالیت آنزیم‌های آنتی‌اکسیدانی و پراکسیداسیون لیپیدی، به ویژه در گربه‌ها انجام نشده است. هدف از این مطالعه بررسی فعالیت برخی آنزیم‌های آنتی‌اکسیدانی، غلظت مالون دی آلدئید (MDA) به عنوان شاخص پراکسیداسیون لیپیدی و ۱۷ بتا استرادیول در سرم قبل و یک ماه بعد از OVH بود. در این مطالعه از ۱۲ گربه ۲ تا ۵ ساله که در دوره‌ی فحلی نبودند، استفاده شد. سپس جراحی OVH انجام شد. نمونه‌ی خون قبل و یک ماه بعد از جراحی گرفته شد و فعالیت آنزیم‌های سوپراکسید دیسموتاز (SOD)، گلوتاتیون پراکسیداز (Gpx) و کاتالاز (CAT)، غلظت MDA و ۱۷ بتا استرادیول در سرم اندازه‌گیری شد. فعالیت SOD، Gpx، CAT و غلظت MDA بعد از OVH نسبت به قبل از آن افزایش یافت، که این افزایش معنی‌دار نبود. غلظت ۱۷ بتا استرادیول پس از عمل نسبت به قبل کاهش یافت که از نظر آماری معنی‌دار بود. OVH پس از یک ماه بدون تغییر قابل توجهی در آنزیم‌های آنتی‌اکسیدانی و پراکسیداسیون لیپیدی در گربه‌ها باعث استرس اکسیداتیو نشد، اگرچه ۱۷ بتا استرادیول کاهش معنی‌داری را نشان داد. برای تعیین ابعاد بیشتر به مطالعات بیشتری نیاز است.

کلمات کلیدی: اواریوهیسترکتومی، آنزیم‌های آنتی‌اکسیدان، پراکسیداسیون لیپیدی، گربه ماده، استرس اکسیداتیو

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