EFFECT OF OVARIOHYSTERECTOMY ON LIPID PEROXIDATION AND LEVELS OF SOME ANTIOXIDANTS AND BIOCHEMICAL PARAMETERS IN BITCHES

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Abstract

The aim of the presented study was to investigate the effect of ovariohysterectomy on lipid peroxidation and levels of some antioxidants and blood parameters in bitches. Thirty non-pregnant healthy bitches (1-3 years of age) were used in the study. All bitches were anaesthetised with xylazine-ketamine. Levels of glucose, total protein, calcium, inorganic phosphorus (IP), alanine amino transferase (ALT), aspartate amino transferase (AST), glutathione (GSH), and malondialdehyde (MDA) were determined in pre-operative and post-operative bitches. The statistical differences were found in the GSH and MDA levels between the pre- and post-operation animals. There were no significant differences of other biochemical parameters. The results of this study showed that the determination of alterations in the GSH and MDA levels could be useful for evaluation of the antioxidant defence mechanism after ovariohysterectomy in bitches.

Key words: bitches, ovariohysterectomy, lipid peroxidation, antioxidants.

Ovariohysterectomy (OVH) is the most common surgical contraceptive in pet animal practice (6, 13). OVH prevents unwanted pregnancy and reduces the risk of development of mammary tumour, pyometra, inconvenience of vaginal discharge, and male attraction during oestrus (8).

Several enzymatic systems are found in tissue to prevent oxidative damage by quenching highly reactive free radicals that initiate lipid peroxidation (24). In healthy animals, the cells devote much of their metabolic activity to the reductive processes that combat the threat of oxidation. Hypothermia, anaesthesia, ischemia of tissues, serous inflammation, and post-operative pain induced by oxidation and lipid peroxidation has been previously described (1, 2, 12, 16).

Glutathione (GSH) is a major intracellular non-enzymatic antioxidant (10) involved in several defence processes against oxidative damage (18, 24). GSH synthesis is regulated by oxidants, antioxidants, and inflammatory and anti-inflammatory agents (20). Malondialdehyde (MDA) level, is a very useful indicator of lipid peroxidation occurring under oxidative stress (28, 29). The plasma MDA concentration is frequently used as a biomarker for an overall lipid peroxidation (17). It was reported that serous inflammation in the peritoneum caused by surgery may also lead to the oxidative stress (7).

The objective of the presented study was to evaluate the effect of OVH on lipid peroxidation and on some antioxidant and biochemical parameters in pre- and post-operative periods in bitches.

Material and Methods

Animals. Thirty non-pregnant clinically healthy mixed-breed bitches, aged 1-3 years, weighing 15-30 kg, were used in the study. They were fed dry feed once a day and water was supplied ad-libitum. The dogs were subjected to clinical examination at the beginning of the trial and healthy dogs were selected. No infection and neoplasm diseases were found in all dogs prior to the operation. No uterine pathological lesions were observed in ultrasonographic examinations.

OVH operation. The bitches were anaesthetised by an injection of xylazine (2 mg/kg b.w., Alfazyne-Alfasan, the Netherlands) and ketamin HCl (15 mg/kg b.w., Alfamine-Alfasan). OVH were performed by medial laparotomy, according to the routine methods (26).

Blood samples were collected into vacutainer tubes with or without heparin (venglect-Terumo Leuven, Belgium) from the saphenous vein of each dog. The samples were centrifuged at 3,000 rpm for 10 min. Blood was collected before beginning of the anaesthesia...
and repeated 24 h after OVH. Blood samples were also taken from pregnant animals once. All analyses were performed on fresh samples.

**Biochemical analysis.** Glucose (Teco Diagnostic, U.S.A), total protein (Diachem LTD, Hungary), calcium, and inorganic phosphorus (Teco Diagnostic, U.S.A) concentrations were determined. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured spectrophotometrically (Schimadzu UV-1601) using commercially available kits (Cormay, Germany). MDA level was determined by using the TBA method as described by Yoshioka et al. (30). MDA, the end product of fatty acid peroxidation, reacts with TBA to form a coloured complex with a maximum absorbance at 532 nm. GSH level was measured as described by Beutler et al. (4). The level of reduced GSH in erythrocytes was determined by the method of Beutler (5). Erythrocytes were firstly deproteinated by addition of trichloroacetic acid (TCA) and then centrifuged (10 min, 3,000 g/min). Thereafter, DTNB [5, 5′-dithiobis (2-nitrobenzoic acid)] was added into the supernatant and the formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25°C against reagent controls.

**Statistical analysis.** The Student’s t-test was used for comparisons between the pre- and post-OVH bitches. All statistical analyses were performed using SPSS statistical programme (Edition 13.0, SPSS Inc., USA). All data were expressed as mean ± SE. P<0.05 was considered as statistically significant.

**Results**

The mean values of investigated parameters and differences between them in the pre- and post-OVH groups are shown in Table 1. When the data of the pre- and post-operation groups were compared; there were significant differences in the GSH (Table 1 and Fig.1) and MDA levels (Table 1 and Fig. 2) (P<0.05). On the other hand, there were no significant differences in calcium, inorganic phosphorus, glucose, and total protein contents, as well as AST and ALT activity between the pre- and post-OVH animals.

**Discussion**

Lipid peroxides are important because their uncontrolled production can result in oxidative stress, with significant damage to cell integrity. Severe oxidative stress can cause cell injury and death (14). Cronauer et al. (7) suggested that serous inflammation in the peritoneum, caused by surgery, may also lead to the oxidative stress.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-OVH</th>
<th>Post-OVH</th>
</tr>
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<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.47 ± 0.24</td>
<td>8.42 ± 0.20</td>
</tr>
<tr>
<td>IP (mg/dl)</td>
<td>6.01 ± 0.28</td>
<td>5.25 ± 0.34</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>61.19 ± 2.26</td>
<td>66.0 ± 3.44</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.35 ± 0.35</td>
<td>5.80 ± 0.42</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>48.35 ± 6.12</td>
<td>52.50 ± 5.15</td>
</tr>
<tr>
<td>ALT (IU)</td>
<td>38.20 ± 3.90</td>
<td>41.61 ± 4.21</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>31.13 ± 2.69*</td>
<td>24.33 ± 2.44*</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>22.30 ± 1.30*</td>
<td>31.59 ± 1.81*</td>
</tr>
</tbody>
</table>

* P<0.05; IP – inorganic phosphorus; ± - standard errors.

**Fig. 1.** Mean GSH levels (mg/dl) of the pre- and post-OVH bitches.

**Fig. 2.** Mean MDA levels (µmol/L) of the pre- and post-OVH bitches.

Some researchers reported an increase in AST activity during 24-72 h after surgical operation (21, 22, 23). In this study, elevated AST activity was found in pre-OVH bitches compared to post-OVH animals 24 h after operation. Our data is consistent with the data of these authors. These findings could be attributed to muscle trauma and tissue damage (11, 22). In the present study, there were no significant differences between pre- and post-operative ALT levels. This situation could be due the ketamine anaesthesia. It was previously suggested that ketamine leads to a moderate oxidative stress without risk of hepatic toxicity (1).
Devitt et al. (9) observed that blood glucose concentrations increased significantly at 1, 2, 4, and 6 h after OVH. Ranganath and Kumar (21) reported that significantly higher glucose level was noticed in bitches during immediate post-operative period. On the other hand, slight increase in glucose level was observed 24 h post-operatively, but the pre-operative and post-operative glucose levels were not significant statistically. High glucose concentration could be connected with an increase in the oxidative stress biomarkers during OVH. According to Benjamin (3), significantly higher blood glucose level in OVH bitches could be due to post-operative stress and pain. There were no significant changes depending on time between pre- and post-operative calcium and total protein concentrations in the presented study. Calcium, inorganic phosphorus, glucose, total protein, AST and ALT were apparently not influenced by the anaesthesia in the current study.

Our data is consistent with data of other authors (15, 23), who showed that the GSH level in plasma in abdominal surgery was 20% lower after operation, compared with the concentration before operation. GSH is involved in several defence processes against oxidative damage (18, 25). Thus it is not surprising that it is expected to be altered under conditions of oxidative stress.

It was stated that (23), anaesthetic drugs and bleeding during operation might induce hypothermia and maintain the thermoregulation muscle activity increase in early post-operative period. Hypothermia, oxidation, and lipid peroxidation have been previously described (1, 23). The results obtained in this study indicated that MDA concentration significantly increased and GSH concentration significantly decreased 24 h after xylazine-ketamine anaesthesia compared with results before OVH. These findings were in agreement with results reported by Serin et al. (23). By contrast, Alva et al. (1) observed no changes in the serum MDA level in rats after xylazine-ketamine anaesthesia. On the other hand, Naziroglu and Gunay (16) observed a significant oxidative effect in dogs subjected to enflurane anaesthesia.

The peritoneum is an organ of high metabolic activity, connected with reactive oxygen species (ROS) generation, and participating in healing process. ROS generated after the trauma caused by surgical intervention leads to damage of cellular components and to lipid peroxidation. Moreover, different inflammatory mediators implicated in oxidative stress, such as hydroperoxides and some cytokines directly induced the de novo synthesis of anti-oxidant enzymes (19, 27). With these observations, the increase might be associated with the alterations in MDA and GSH levels after OVH.

In conclusion, this study demonstrated that the level of GSH significantly decreased, whereas MDA concentration significantly increased in bitches after OVH, performed under xylazine-ketamine anaesthesia. The determination of GSH and MDA levels could be a useful tool for evaluation of anaesthetic and surgical side effects after OVH in bitches. Moreover, this allows deeper understanding of oxidative stress mechanism in these pathologies.

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References


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