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Effect of en bloc ovariohysterectomy on Th1/Th2 cytokine balance and organ histopathology in rats

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Summary

The aim of this study was to investigate the effects of en bloc ovariohysterectomy on the Th1/Th2 cytokine balance, as well as on visceral organ and brain histopathology in rats. A total of 28 Sprague Dawley female rats aged 3-4 months and weighing 200-250 grams were used in the study. Fourteen of them were pregnant. The 14 non-pregnant rats were divided into two groups: the control group (Group 1, n: 7) and the ovariohysterectomized group (Group 2, n: 7). All rats underwent en bloc ovariohysterectomy on gestational day 20-21. The 14 pregnant rats were divided into two groups: those with live infants were grouped as Group 3 and those with dead infants were grouped as Group 4. All the rats (n: 28) were decapitated at the end of one month, blood samples were obtained, and the organs were isolated. The Th1 [interleukin 2 (IL-2), tumor necrosis factor alpha (TNFα)] and Th2 [interleukin 4 (IL-4), and interleukin 10 (IL-10)] levels in the blood sera were measured by the ELISA method, and histopathological analysis was performed on the isolated tissues. The differences between the groups were found to be insignificant with regard to IL-2, IL-4, and TNFα levels ($p > 0.05$). However, a significant difference was observed for IL-10 levels between Groups 3 and 4 ($p < 0.05$), and the highest IL-10 level (150.32 ± 71.64 pg/ml) was determined in Group 4. No important pathological findings were observed in the cardiac and brain tissues of any of the animals in the histopathological examination. Inflammatory changes were observed in the pulmonary and renal tissues of the rats in Groups 2, 3, and 4, where the changes were commonly interstitial pneumonia in Groups 2 and 4, and interstitial nephritis in Group 3. According to the findings of this study, en bloc and standard ovariohysterectomies performed in rats had similar effects on the Th1/Th2 cytokine balance and the histopathology of the brain and visceral organs.

Keywords: en bloc ovariohysterectomy, Th1/Th2 cytokine, histopathology, rats

Different methods have been used to control reproduction via operative interventions in cats and dogs. These include ovariohysterectomy, ovariectomy, hysterectomy, salpingectomy, and *en bloc* ovariohysterectomy. The method selection depends on the choice of the owner, the health condition of the animal, and the economic status of the owner (8).

En bloc ovariohysterectomy is performed particularly in animals for which cesarean section is indicated because of the risk of a difficult delivery. This technique includes the application of ovariohysterectomy while the fetuses are still in the uterus. *En bloc* ovariohysterectomy may be performed whether the fetuses are alive or dead, and this method permanently blocks the reproductive ability without the need for a second operation. Besides, ovariohysterectomy is performed on a compulsory basis during cesarean section in cases of fetal death, putrefaction, gangrene, or toxemia (1, 20).

The survival rate of the newborn in dogs has been reported as 75% for cesarean sections performed by the *en bloc* technique (12), and 92% for sections performed by the conventional method (7, 13). If the uterus is to be surgically removed, ovariohysterectomy is recommended after the delivery for better survival of the newborns (1, 6, 20).

The *en bloc* method is not recommended in the case of bradycardic or hypoxic fetuses due to suppressed or impaired blood circulation to the uterus. When this operation is performed on a uterus containing live fetuses, the time between the first ligature performed on the vessel and the delivery of the infants should be shorter than 60 seconds (20).

Cytokines secreted from cells, particularly $CD4^+$ T lymphocytes, have important roles in the regulation of immunological reactions. Th0 lymphocytes are differentiated into two subgroups as Th1 and Th2: IL-2, IL-12, IL-15, IL-18, IFNγ, and TNFβ are secreted from the Th1 sub-group of $CD4^+$ T lymphocytes, whereas IL-4, IL-5, IL-6, IL-10, IL-13, and GM-CSF are secreted from the Th2 sub-group of $CD4^+$ T lymphocytes. Th1 cells are responsible for cellular immunity, whereas Th2 cells are responsible for humoral immunity. Macrophages and NK cells are the cells of natural immunity, and they affect the functional polarization of $CD4^+$ T lymphocytes by producing essential cytokines. IFNγ and IL-12 are necessary for the polarization of Th1 cells, and IL-4 is necessary for the polarization of Th2 cells (2, 14, 16).

Many studies have been conducted on the effects of ovariohysterectomy on cats and dogs. However, there is no study in the literature on the effects of *en bloc* ovariohysterectomy, which is technically and periodically different from standard ovariohysterectomy, on the Th1/Th2 cytokine balance and the histopathology of visceral organs. Therefore, the aim of this study was to investigate the effect of *en bloc* ovariohysterectomy on the Th1/Th2 cytokine balance and visceral organ histopathology in rats.

Material and methods

A total of 28 Sprague Dawley female rats weighing 200--250 grams and aged 3-4 months were used in the study, and among these 14 were pregnant. The animals were provided by the Experimental Research Center of Firat University. They were kept in individual cages throughout the study, exposed to a 12/12 hour light/dark cycle, and fed *ad libitum.* The study was approved by the Experimental Animals Local Ethical Committee of Firat University (Report no: 10.02.2016 – 2016/11).

The animals were grouped as follows:

Group 1: control group – animals that had not undergone ovariohysterectomy (n = 7).

Group 2: animals that were not pregnant and had undergone ovariohysterectomy (n = 7).

Group 3 (n = 7): animals that were gestational day 20-21, had undergone *en bloc* ovariohysterectomy, and had live infants.

Group 4 (n = 7): animals that were gestational day 20-21, had undergone *en bloc* ovariohysterectomy, and had dead infants (These infants died spontaneously – probably because their mothers were not interested).

The sexual cycle and the gestational timing of the animals were determined by a method described by Risvanli et al. (15). In this method, vaginal irrigations were performed with sterile distilled water by means of elastic pipettes and tips. The fluid obtained by irrigation was placed onto slides and examined under 40X magnification of the microscope, and the density of the cell types were scored as +, ++, and +++. Animals with a superficial cellular density of +++ were accepted to be in their estrus period. Animals with a visualized spermatozoid on the slides prepared with vaginal irrigations were accepted to have had coitus, and that day was recorded as the 0th day of gestation.

Rompun (10 mg/kg IM) – Ketalar (90 mg/kg IM) combination anesthesia was administered to the rats during the operations. Following the routine procedures, e*n bloc* ovariohysterectomy was performed on the animals on gestational day 20-21. Subsequently, the infants within the uterus were delivered and placed beside their mothers. Standard ovariohysterectomy was performed on the animals in Group 2, which were not pregnant (1).

Cytokine analysis. All animals were decapitated at the end of the post-operational first month, their blood samples were obtained, and their organs were removed. Sera obtained from these blood samples were kept at –80°C until cytokine analysis. The test procedure described in Commercial ELISA (Bio-X Diagnostics Jemelle, Belgium) kits was performed in order to measure IL-2, IL-4, IL-10, and TNFα concentrations in the sera, and the results were read by the ELISA reader (BİO-TEK ELX800) at 450 nm wavelength (9, 17).

Histopathological tests. Cerebral, cardiac, pulmonary, hepatic, and renal tissue samples were obtained from all animals that had been necropsied, and fixed in 10% buffered formaldehyde solution. Following the routine procedures, the tissues were prepared as paraffin blocks, and sections of 5 micron thickness were obtained. Hematoxylin-eosin staining was added, and the sections were then examined under a light microscope.

Statistical analysis. One rat in Group 4 died during the data follow-up in the study, and so was excluded from the study. The end-analysis of the study included six rats in Group 4 and seven rats in each of the remaining groups. The data of 27 rats were utilized. Descriptive statistics were used for the analysis of IL-2, IL-4, IL-10, and TNFα concentrations in serum samples. The Kruskall Wallis variance analysis was used for comparisons between the groups with regard to the same parameters, and the Bonferroni Mann Whitney U test was used for the parameter with significance in the follow-up. These analyses were performed using the SPSS package program (18).

Results and discussion

The descriptive values of IL-2, IL-4, IL-10, and TNFα in blood samples and the comparative analysis of the groups are presented in Tab. 1. No significant difference was observed between the groups with regard to IL-2, IL-4, and TNFα levels ($p > 0.05$). However, a significant difference was observed between Groups 3 and 4 with regard to IL-10 levels ($p < 0.05$), and the highest IL-10 level was determined in Group 4 (150.32 ± 71.64 pg/mL) (Tab. 1).

Histopathologically, no important microscopic lesions were observed in the cardiac or cerebral tissues in any of the rats. Inflammatory changes were observed in the pulmonary, renal, and hepatic tissues of the rats in Groups 2, 3, and 4, and these changes are shown in Tab. 2. Focal and mild mononuclear infiltra-

Tab. 1. Descriptive values of IL-2, IL-4, IL-10, and TNFα in the blood samples of the rats, and group comparisons (n = 7)

Groups	Statistic measures	IL-2 pg/mL	IL-4 pg/mL	IL-10 pg/mL	TNFα pg/mL
Group 1	Mean	97.76	5.12	74.76^{AB}	61.16
	Std. Deviation	43.86	2.24	50.58	16.94
	Median	77.96	6.72	57.11	69.50
	Minimum	59.42	2.41	33.03	39.10
	Maximum	182.23	7.20	173.26	76.63
Group 2	Mean	104.21	5.03	111.79^{AB}	78.69
	Std. Deviation	59.34	2.68	62.80	25.27
	Median	65.03	3.68	93.36	70.23
	Minimum	61.81	2.41	54.59	63.06
	Maximum	194.83	9.20	236.55	134.92
Group 3	Mean	81.16	5.73	67.51^{A}	61.93
	Std. Deviation	19.43	1.93	23.54	16.16
	Median	73.68	6.67	66.44	68.78
	Minimum	62.50	3.26	34.13	39.10
	Maximum	115.68	8.17	104.89	77.13
Group 4	Mean	114.77	4.69	150.32^{B}	71.40
	Std. Deviation	24.71	1.99	71.64	14.99
	Median	104.18	3.83	135.21	74.89
	Minimum	92.49	2.96	69.65	42.53
	Maximum	159.29	7.30	237.47	84.21
Kruskal Wallis Test		3.546	1.014	8.407	3.145
P		0.315	0.798	0.038	0.370

Explanation: Means with different superscript letters in the same column differ significantly at $p < 0.01$.

Tab. 2. Number of cases with inflammatory changes in visceral and brain tissues

Groups	Inflammatory cell infiltration				
	Liver	Kidneys	Lungs	Heart	Brain
Group 1	0/7	0/7	0/7	0/7	0/7
Group 2	0/7	1/7	4/7	0/7	0/7
Group 3	1/7	5/7	1/7	0/7	0/7
Group 4	1/6	1/6	4/6	0/6	0/6

tion was observed in the portal areas in the liver of one rat in both Group 3 and Group 4. Pulmonary and renal inflammatory changes were commonly observed in rats in groups other than the control group, and renal multifocal, interstitial mononuclear cell infiltration and mild edema were observed in Groups 2 and 4. Interstitial pneumonia was notable in rats in Group 3. Thickenings were observed in the intra-alveolar regions of the pulmonary tissues of these animals due to mononuclear cell infiltration and mild edema.

The literature lacks information on the possible effects of the e*n bloc* ovariohysterectomy method, which is frequently used in the gynecology of cats and dogs, on the post-operative lives and health of these animals.

It has been reported in a study on rats (11) that ovariohysterectomy may cause morphological changes in the lymphoid organs and peripheral blood in the long term, but it does not have much effect on antibody production.

It has also been reported that acute phase proteins increase as a result of an increase in the concentrations of pro-inflammatory cytokines (IL-1, IL-6, and TNFα) in blood due to various traumas (4). On the other hand, IL-10 is known to be a major suppressor of the immune response and inflammatory activity by inhibiting the synthesis of pro-inflammatory cytokines (10). It has been suggested that ovariohysterectomy increases the IL-6 and IL-10 production capacity of the leukocytes, but has no such effect on TNFα. These results indicate that an ovariohysterectomy operation may have systemic effects by inducing the cytokine secretion of leukocytes in circulation (21).

It has been reported that surgical interventions performed on dogs increase the number of polymorphonuclear neutrophils in the circulation and thereby increase the concentration of inflammatory cytokines (22). Dąbrowski et al. (5) conducted research on ovariohysterectomized dogs with pyometra and reported that IL-6 and IL-10 concentrations decreased after the operation, but increased on the third post-operative day in healthy dogs. In another study on dogs, unchanged blood IL-10 concentrations were reported within 24 hours of an ovariohysterectomy operation (10). In the present study, no significant differences were observed in IL-2, IL-4, and TNFα levels between the groups ($p > 0.05$). However, when the IL-10 levels were investigated, the difference between Groups 3 and 4 was found to be significant ($p < 0.05$), and the IL-10 concentration was the highest in Group 4. However, a difference in IL-10 alone, without a difference in IL-6, was not evaluated as a shift in the Th1/Th2 cytokine balance in favor of Th2.

The neuroprotective effect of estradiol has been demonstrated in different studies. It has therefore been suggested that exogenous natural and synthetic applications might be useful in preventing possible problems, especially in the nervous system and brain of female rats (3, 6, 19). Other studies have suggested that low doses of estradiol (10-25 pg/ml) administered just after ovariectomy prevent global ischemia-related brain damage in young and middle-aged rats, and focal ischemia-related brain damage in young rats. However, this was not the case when estradiol was administered 10 weeks after ovariectomy (19, 23). In the present study, no pathology was observed in the cerebral or cardiac tissues one month after the operations in any of the groups, but inflammatory changes were observed in the pulmonary and renal tissues of the animals.

According to the findings of this study, *en bloc* and standard ovariohysterectomies performed in rats have similar effects on the Th1/Th2 cytokine balance and the histopathology of the liver, kidneys, and lungs.

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