

# The Effect of Canine Ovariohysterectomy on HSP70 and Anti-HSP70 Antibodies <sup>[1]</sup>

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## Summary

The present study aimed to determine the effects of ovariohysterectomy on blood serum concentrations of Heat Shock Protein (HSP) 70 and anti-HSP70 antibodies in dogs. For this purpose, 87 female stray dogs were used as the materials. Ten milliliters of blood was taken from the animals preoperatively and on the first day postoperatively. The concentration of extracellular HSP70 and anti-HSP70 antibodies in blood serums were measured by using commercial ELISA kits. Subsequently, the preoperatively and postoperatively obtained data were compared. As a result, the mean +/- SD concentration of HSP70 (4.86±0.99 ng/mL) and anti-HSP70 (109.77±16.64 ng/mL) antibodies in the dogs' blood samples taken after ovariohysterectomy were found to be significantly lower when compared to the preoperative amounts.

**Keywords:** HSP70, Anti-HSP70, Ovariohysterectomy, Dog

## Köpeklerde Ovaryohistektomi'nin HSP70 ve Anti-HSP70 Antikorları Üzerine Etkisi

### Özet

Bu çalışmada köpeklerde kan serumu Heat Shock Protein (HSP) 70 ve anti-HSP 70 antikorlarının düzeyleri üzerine ovaryohistektominin etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla, 87 adet dişi sokak köpeği materyal olarak kullanıldı. Hayvanlardan operasyon öncesi ve sonrası 1. gün 10 ml kan örnekleri alındı. Kan serumlarındaki hücre dışı HSP70 ve anti-HSP70 antikorlarının miktarları ticari ELISA kitleri kullanılarak ölçüldü. Daha sonra operasyon öncesi ve sonrası elde edilen verilerin istatistikî analizleri yapıldı. Sonuç olarak; köpeklerde ovaryohistektomi sonrası alınan kan numunelerindeki HSP70 (4.86±0.99 ng/ml) ve anti-HSP70 (109.77±16.64 ng/ml) antikorlarının miktarlarının operasyon öncesine göre daha düşük olduğu tespit edildi.

**Anahtar sözcükler:** HSP70, Anti-HSP70, Ovaryohistektomi, Köpek

## INTRODUCTION

Ovariohysterectomy is a surgical operation frequently used to control reproduction dogs. Following the operation, some complications may develop in dogs such as obesity, cardiac stress, urinary incontinence, hair loss, and change of temper, infection of the operation site, peritonitis, colonic and urethral obstructions developing due to peritoneal adhesions, stump pyometra, and ovarian remnant syndrome <sup>1</sup>. Although one can take preoperative and postoperative

precautions to prevent such complications, there is always a possibility that they may develop. Therefore, there is a need for novel research about the causes of and solutions for such complications and postoperative development of infections in particular. At this point, correct assessment of the state of a dog's immune system prior to and following ovariohysterectomy is important to prevent complications or at least, to identify their causes.



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Recent years have witnessed an increase in the number of studies on HSPs in dogs as well as in other living organisms. Various publications have shed light on the relationship of these proteins with the immune system <sup>2,3</sup>.

Heat Shock Proteins enhance a cell's resistance to stress. In cases of cell damage occurring under stressful conditions, there is an increase in the amount of these proteins, which serve in the process of renewing damaged cells <sup>4,5</sup>. The amount of HSPs increases due to high temperature, fever, inflammation, ischemia, hypertrophy, cellular damage, diseases like cancer, and cellular interactions <sup>4,6</sup>.

Heat Shock Proteins are molecules that help to many antigens induce an immune response in the host. Moreover, these proteins are also believed to assist the formation of molecules that allow the immune system to identify diseased or normal cells. Appearing as a result of immune responses against these proteins, anti-HSP70 antibodies may also cause the development of a reaction against the cell itself. These antibodies may also cause autoimmune diseases <sup>7,8</sup>.

Heat Shock Proteins are grouped in five according to their molecular weight, structure, and functions. These groups include HSP100, HSP90, HSP70, HSP60, and small heat shock proteins <sup>4</sup>. Heat Shock Protein 70 functions in intracellular protein delivery. It protects stressed proteins, prevents the aggregation of unfolded proteins, and binds polypeptides. This molecule is found at tissue level as well as in serum <sup>4,9</sup>.

Intracellular HSP70 has a cell protective effect and serves in the development of ischemic tolerance in tissues like the brain <sup>10,11</sup>. It has been shown that in cases which induce sublethal cellular stress such as hypothermia or ischemia, intracellular HSP70 plays a significant role in rats, rabbits, mice, and dogs <sup>12</sup>.

In this study was aimed to determine the effects of ovariectomy on blood serum levels of HSP 70 and anti-HSP 70 antibodies in dogs.

## MATERIAL and METHODS

The materials used in this project included 87 healthy female stray animals of various breeds and at varying ages (8 months - 6 years, nonpregnant) brought for ovariectomy to the Small Animals Clinic in the Animal Hospital at the Veterinary Faculty of Firat University. An Ethical Board Report was obtained from the Ethical Board for Experimental Animals at Firat University to conduct the study (2011/3-51).

Xylazine (0.1-0.8 mg/kg IV)/Ketamin HCl (7-10 mg/kg, IM) anesthesia was performed for ovariectomy. Following a mid-line incision, the ovaries and uterus were removed. As a preventive measure against following surgery complications, a parenteral antibiotic (Enrofloxacin, 2.5 mg/kg IM) was administered. The analgesic drug (Meloxicam,

0.2 mg/kg IM) was used postoperative.

10 ml of blood was taken from the animals preoperatively and on the first day postoperatively (after 24 h). The blood samples used in the study has been not taken especially for this study. Also, the blood samples were used from the examples that routinely taken before and after operations. This application has to reveal for the hematological status of animals. The blood samples were taken from anesthetized animals before the operation. Following routine procedures, the blood samples were separated into serums, which were kept at -80°C until the measurements.

Commercial ELISA kits were used to measure the amounts of extracellular HSP70 (Uscn Life Sciences, PRC) and anti-HSP70 (Enzo Life Sciences, USA) antibodies in the blood serums <sup>13,14</sup>. Next, the pre- and postoperatively-obtained data were subjected to statistical analyses for comparison.

The statistical analyses were performed by using the Wilcoxon test in SPSS 11.5 software.

## RESULTS

No complications occurred in animals after operations. *Table 1* summarizes the data obtained from blood the samples collected pre- and postoperatively. Thus, the amounts of HSP70 ( $4.86 \pm 0.99$  ng/mL) and anti-HSP70 ( $109.77 \pm 16.64$  ng/mL) in the blood serums after the ovariohysterectomy were found to be lower when compared to preoperative amounts ( $7.22 \pm 1.30$  ng/mL,  $143.67 \pm 20.81$  ng/mL) ( $P < 0.01$ ).

**Table 1.** Anti-HSP70 and HSP70 concentrations before and after operation.

**Tablo 1.** Operasyon öncesi ve sonrası Anti-HSP70 ve HSP70 konsantrasyonları

Parameter	Preoperation (n=87)	Postoperation (n=87)	P
HSP70 (ng/mL)	7.22±1.30 <sup>a</sup>	4.86±0.99 <sup>b</sup>	*
Anti-HSP70 (ng/mL)	143.67±20.81 <sup>a</sup>	109.77±16.64 <sup>b</sup>	*

\*  $P < 0.01$ , <sup>ab</sup> The difference between different letter carrying averages is significant

## DISCUSSION

Increased HSP concentrations in cells exposed to stress are observed as being protective against possible future damages <sup>15</sup>. Under physiologic conditions, HSPs play roles in important activities for cell functions. Among them, delivery and configuration of newly-synthesized proteins occupy a significant place. HSPs have crucial roles in ensuring the survival of cells and their protection against harmful effects under hyperthermia or other (oxidative, toxic, osmotic, hypothermic, etc.) stress types and HSP70 is an important HSP that is increased under the abovementioned stress conditions <sup>16,17</sup>. Heat Shock Protein 70 has a direct role in protection against ischemia-reperfusion damages <sup>18,19</sup>. The amounts of HSP70-related proteins and mRNA are known to

increase in dogs following ischemias developing in organs like the heart. In such cases, HSP70 has been proposed to provide protection against ischemic damages<sup>19,20</sup>.

Canine HSP70 gene has a 90-95 percent of sequence similarity to bovine, human, and mouse HSP70 proteins<sup>21</sup>. Accordingly, in the present study, concentrations of canine HSP70 and anti-HSP70 antibodies were measured by using human ELISA kits.

Heat Shock Proteins are immunogenic<sup>22</sup>. Therefore, antibodies developing against HSPs were identified in the blood serums of normal individuals<sup>23,24</sup>. Anti-HSP70 antibodies are known to be autoantibodies naturally found in the blood circulation of healthy pregnant women. However, it is also suggested that further research should be conducted to investigate the relationship of normal pregnancy and the immunobiology of preeclampsia with the antibodies against HSPs<sup>25</sup>.

Oxidative stress is increased during and following surgical operations depending on reactive oxygen species and related cytokines, leading to the activation of Heat Shock reactions, which results in an increase in HSPs depending on the intensity of stimulation<sup>26</sup>. Yet, following such interventions, different cases may also occur due to the negative feedback responses of Heat Shock reactions, regardless of systemic inflammatory response. Accordingly, a significant postoperative decrease in HSP70 has been reported in humans following serious surgical interventions. It is also reported that after such operations, HSP70 concentration rapidly declines to the lowest levels during the postoperative period; therefore, HSP70 can be used as a postoperative prognostic marker. Similarly, a decreased amount of postoperative intracellular HSP70 is in parallel to the decrease in the amount of autoantibodies in blood circulation<sup>27</sup>. In this study, we also found that the dogs had lower amounts of HSP70 and anti-HSP70 in their blood serums following ovariectomy when compared to the preoperative amounts.

Following acute ischemias, the amounts of anti-HSP70 antibodies also increase due to increased amounts of HSP70. Greater amounts of Heat Shock Protein 70 proteins in blood circulation may also cause an increase in the amounts anti-HSP70 antibodies<sup>28</sup>.

Leng et al.<sup>29</sup> reported that the rates of anti-HSP70 antibodies increased in atherosclerosis-induced rats. Quijada et al.<sup>30</sup> argued that the amount of anti-HSP70 antibodies does not increase during canine Leishmania infections.

It has been reported that ovariectomy in rats significantly elevated HSP70 concentrations in left ventricular cell lysates<sup>31</sup>. However, this view has been rejected in some studies. For instance, the researchers in a study observed a significant down-regulation of HSP72 in the heart muscles of ovariectomized rats<sup>32</sup>. Similarly, ovariectomy in aged mice has been claimed to lower the level of HSP70 proteins in the brain,

when compared to younger animals. This study reported that estrogen deficiency due to aging down-regulates brain HSP70 expression and that this hormone has significant effects upon the regulation of HSP release in the brain<sup>33</sup>. In the present study, we also found that the amounts of HSP70 ( $4.86 \pm 0.99$  ng/ml) and anti-HSP70 ( $109.77 \pm 16.64$  ng/ml) in canine blood serum following ovariectomy were lower than the preoperative levels (Table 1).

In the light of the study's results, the amounts of HSP70 and thus anti-HSP70 antibodies in canine blood serum following ovariectomy were found to be lower than the preoperative amounts. The relationship of HSPs with immune system has been shown in dogs as well as other species. This has led to the hypothesis that ovariectomy reduces HSP70 concentrations in dogs, which weakens the immune system and precipitates the development of various postoperative complications. Nevertheless, this conclusion needs to be confirmed by further studies.

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