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Research Article

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Comparison of the Effect of Ovariectomy and Ovariohysterctomy on Some Immunity Responses in Dogs

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ABSTRACT

Ovariohysterectomy and ovariectomy are commonly used to control the pet population and the health and behavioral benefits of dogs and cats. The aim of this study was to evaluate the inherent and humoral immune factors following ovariohysterectomy and ovariectomy surgery in female dogs. In this study, 18 female dogs were used. After anesthesia, animals received one of the following three treatments randomly: 1- laparotomy (control group) 2- ovariohysterectomy 3- ovariectomy. The intrinsic and humoral immunity at baseline, 7, 14, 30 and 60 days after surgery were evaluated. In the control group, up to the 7th day of the study, serum myeloperoxidase and bactericidal activity increased, lysozyme and complement activity decreased, and constant antitrypsin activity was observed. Ovariohysterectomy and / or ovariectomy have led to a change in the pattern of variation in the experimental group. Except for antitrypsin, the change in the other factors compared to the control group was milder or less; there was a significant difference in the activity of the complement, myeloperoxidase and lysozyme activity compared to the control group. The total amount of immunoglobulins in the blood, the level of anti-salmonella antigen and anti-sheep red blood cell antibody in the experimental groups was decreased in comparison to the control on day 7. Although ovariohysterectomy and ovariectomy resulted in a weaker immune response than the control group, there was no significant difference between the two groups.

Key words: *Dog, Ovariohysterectomy, Ovariectomy, Immune System.*

INTRODUCTION

Ovariohysterctomy is commonly used to control the population of pets and the health and behavioral benefits in dogs and cats [1]. It has been shown that animal sterilization reduces reproductive system problems, such as mammary tumors [2] and pyometra [3]. Recent studies have shown that sterilization may cause health impairment, including changes in the incidence of cancer, orthopedic problems, and immune system impairment [2-5]. For example, it has been shown that the probability of transcranial cell carcinoma (TCC) in bladder increases by three times after the sterilization surgery. Moreover, hegemon sarcoma, osteosarcoma and lymphosarcoma have been high in sterilized animals than those in non-sterilized animals [2]. It has been stated that sterilization may be associated with immunosuppression-related diseases such as myasthenia gravis [6, 7] and immune-mediated hemolytic anemia [8]. Female dogs can be sterilized using ovariohysterctomy or ovariectomy methods. Although ovariohysterctomy is still used as a common method for sterilizing female dogs, ovariectomy has some advantages such as smaller cutting, better observation of the ovarian base and less risk of complications caused by the manipulation of the uterus surgery [1]. The immune system in humans and animals includes both central (primary) and peripheral (secondary) immune organs plus circulating cells in the

blood [9]. The immune system is known to be very sensitive to changes in the body and general health [10]. The immune system of dogs might also show a different response to ovariohysterctomy and ovariectomy. Based on the authors' knowledge, no study has been conducted so far to examine and compare the effect of ovariohysterctomy and ovariectomy on the immune function of dogs. Thus, the present study was conducted with the aim of evaluating some intrinsic and humoral immune factors following ovariohysterctomy and ovariectomy in female dogs.

MATERIAL AND METHODS

In the present study, 18 female dogs with the mean age of 1.5-2.5 years based on dental evaluation having the same weight were used. Animals' health status was confirmed by the clinical examination and CBC and TP measurements. During the study, animals had access to water and food twice per day. 12-hour food abstinence and 2-hour water abstinence were applied to each animal before the study. It should be noted that all of the surgeries took place in the anestrus period. To evaluate the estrus cycle, smear was prepared from all groups before the study. After transferring dogs to the study site, animals were given 30 minutes to be adapted to the environment. Animals received acepromazine (0.1 mg / kg) and morphine (0.4 mg / kg) in a single syringe in the left leg hamstring muscles. Thirty minutes after the sedative injection, the animals were transferred to the table, and the vein of the left and right hands was catheterized. Then, 100% oxygen was placed on the animal for 5 minutes with a mask. Anesthesia was induced with propofol (6 mg / kg). After the induction of anesthesia, the trachea of animals was intubated and connected into oxygen. Anesthesia was maintained with propofol (0.3 mg / kg). The animals were placed in the supine position, and the surgery site was prepared. The animals were randomly assigned to one of the following three treatments:

1. Laparoscopic surgery including opening the abdominal area and closing it (Control)
2. Ovariohysterctomy including ligaturing ovarian bases and the uterus body (OHE)
3. Ovariectomy including ligaturing ovarian from both sides and removing them (OVE)

All surgeries were performed by a single person. The Fossum (2013) method was used in ovariohysterctomy and ovariectomy surgeries [11]. The animals received normal saline (10mg / kgh) throughout the surgery intravenously, and received 100% oxygen through a tracheal tube. The heart rate, respiratory rate, anal temperature of the animals were measured and recorded before anesthesia induction, during the anesthesia, and during the recovery. Moreover, the dose of anesthetic drug administrated, and the duration of anesthesia and surgery was recorded. After the surgery, all dogs received cefazolin (22 mg / kg) and tramadol (mg / kg 1) for 3 days.

Evaluation of immune system activity

The level of intrinsic and humoral immune activity was evaluated at baseline, 7, 14, 30 and 60 days after the surgery.

Intrinsic immunity evaluation

Serum bactericide

First, E. coli and Staphylococcus aureus bacteria were cultured in TSA medium, and the plates were placed in a 37 ° C incubator. After observing that the bacteria were grown, the colonies of each plate were removed with sterilized needle and mixed with sterile PBS, and its turbidity was brought to 1 McFarland. Then, in a 96-well plate, 25 µl of sterile PBS was added to all wells, and 25 µl of active and inactive serums were added to each of the wells so that active serums were poured to one column, and the inactive serums were poured in the adjunct column at the same time (to deactivate the serums, they were placed in Ben Murray at 56 ° C for 30 minutes). This procedure was done for all active and inactive serums at any time, and finally, 50 µl of bacterium diluted with sterile PBS was added to all the wells. The plate was kept in an incubator at 37 ° C for 90 minutes. Then, 50 µl of TSB medium was added to all of the wells, and their light absorption was read at 600 nm. The plate was placed in an incubator at 37 ° C for 4 hours, and then, the light absorption was read at 600 nm. It should be noted that several wells were considered as controls. In two wells, 50 µl of PBS and 50 µl of Escherichia coli and Staphylococcus aureus were added, and 50 µl of PBS and 50 µl of TSB medium were added to the other wells. Finally, 50 µl of PBS, 50 µl of TSB medium and 50 µl of the mentioned were added to two wells. The serum bactericidal activity in the studied groups was calculated by comparing the differences in the light absorption measured in active and inactive sera, and the results were interpreted according to the level of bacteria growth inhibition.

Evaluating the complement secondary pathway activity

In order to evaluate the complement pathway activity, 50 µl of active and inactive serums were poured in sterile microtubules separately at different times. Then, 350 µl of PBS containing Ca2 + and Mg2 + were poured into each micro-tube, and finally, 100 µl of Rabbit rinsed red blood cell 5% was added to each micro-tube, and the micro-tubes were placed in an incubator at 37 C for 45 minutes. Then, micro-tubes were centrifuged at 3000 rpm for 5 minutes, and 100 µl of supernatant fluid of each microtube was collected and poured into a 96-well micro-plate plate so that the active serum was poured into one column of a specific time, and the inactive serum was poured into an adjunct column at the same time. This was done for all serums and at all times, and finally, the light absorption was read at wavelength of 450 nm. Standard samples were prepared by adding 0, 25, 50, 75 and 100 µl of rabbit degraded red blood cells and brining the volume to 100 µl using PBS. The percentage of degradation of red blood cells in each sample was calculated in comparison with the light absorption of the standard samples.

Evaluating the complement classic pathway activity

The inactive serum of rabbits immunized with sheep red blood was kept at a concentration of 25% with red blood cell 10% of an adjacent sheep for 24 hours at 4 ° C. Then, it was rinsed with PBS and its supernatant was removed. 25 µl of active serum plus 375 µl of complement buffer as well as 100 µl of sheep red blood cells 5% were poured into microtube and placed in the incubator at 37 C for 45 minutes. Then, it was centrifuged for 5 minutes with 3000 rpm, and 100 µl of the supernatant of each serum was collected and poured into 96-well plate of ELISA. Finally, the light absorption of the samples was read at 490 nm. Standard samples were prepared by adding 0, 25, 50, 75 and 100 µl of the sheep degraded red blood cells and bringing the volume to 100 µl using PBS. The rate of the degradation of the red blood cells in each sample was calculated in comparison with the light absorption of standard samples.

Evaluation of lysozyme activity

20 µl of serum samples were poured into a 96-well plate so that the active serum was poured in a column at a specific time, and the inactive serums were poured into adjunct column at the same time. Then, 80 µl of Micrococcus lysodicaccus + sodium acetate buffer (pH =6) was added to the wells, and in wells of the column, as control, only 100 µl of the mixture of bacterium and lysozyme buffer was added, and finally, light absorption of the samples was read after 5 minutes at 490 nm. The activity level of lysozyme was calculated as a unit of activity in each minute per one milliliter of serum [12].

Evaluation of myeloperoxidase activity

15 µl serum was mixed with 135 µL of HBSS buffer and 25 µl of TMB 20 mM + 25 µl of H_2O_2 20 mM was added to the microtubule, and after 2 minutes, 50 µl of H_2SO_4 (4 M) was added to the contents of the micro-tubes, and then, the micro-tubes were centrifuged (5 min with 8000 rpm). Finally, 100 µl of the light absorption of the supernatant was read at 450 nm [13].

Evaluation of antitrypsin activity

10 µl of serum was added to 100 µl of Tris-HCl solution (100 µl of 50 mM pH 8.2) containing 20 g of trypsin. In the serum control sample, 10 µl of serum was added to 100 µl of Tris-HCl solution, and the positive control sample included Tris-HCl solution containing 20 g trypsin without serum. The samples were brought to 200 µl by adding Tris-HCl and placed at room temperature for one hour. Then, 2 ml of BAPNA (Na-benzoyl-DL-arginine-p-nitroanilide HCl) substrate at concentration of 0.1 mM in Tris-HCl and 20 mM calcium chloride were added to all samples. After 15 minutes, the reaction was stopped by adding 500 µl of acetic acid 30%, and the light absorption was measured at the wavelength of 405 nm. The percentage of trypsin inhibition was calculated by calculating the differences between the wavelengths of the serum-free positive control samples.

Evaluation of humoral immunity

In this study, the total antibody level was evaluated by zinc sulfate sedimentation method, and the antibody level in response to subcutaneous administration of sheep red blood cell (10%), bovine serum albumin (1 mg / kg), and killed Salmonella typhimurium bacterium. One milliliter of antigen in sterile solution of NaCl 0.15 mol/L was administrated 5 days before the anesthesia. Blood sampling was performed to evaluate the level of produced antibodies relative to the injectable antigens by hemagglutination and microagglutination method.

Measurement of total serum immunoglobulin

Zinc sulfate sedimentation method was used in this regard. First, 0.7 millimolar of buffer of zinc sulfate was prepared, and pH was set on 5.8. Then, 12.5 µl of the serums at times of zero, 5 days, and 10 days after the

surgery were poured separately in sterile micro-tubes and 850 µl of zinc sulfate was added to each micro-tube and mixed well. After placing at room temperature for 2 hours, 100 µl of the above solution was poured into a 96-well plate, and the light absorption was read at a wavelength of 600 nm [14].

Evaluation of anti-salmonella antibody level

First, 50 µl of PBS containing 0.05% phenol was added to each of the feeds wells (in the first row of the plate, 75 µl of PBS was added instead of 50 µl), and then, 25 µl of serum was poured into the first well, and the dilution was done and the serum was not added to the wells of the last row. Then, 50 µl of antigen (Salmonella typhimurium bacterium) was added to all wells. The bacterium was deactivated before at 95 ° C Ben Murray for one hour, and the antigen concentration was brought to similar turbidity of the standard McFarland standard 4. Then, the plates were placed at 37 ° C incubator for 24 hours, and the results were read.

Evaluation of anti-red blood cell antibody level

First, 50 µl of PBS was added to all of the wells (in the first row of the plate, 75 µl of PBS was added instead of 50 µl). Then, 25 µl of the serum was poured into the first well, the dilution was performed and the serum was not added to the last row wells. Then, 50 µl of the sheep red blood cells (1%) was added. Then, the plates were placed at room temperature for 2 hours, and finally, the results were read.

Statistical analysis

The statistical analysis of the present study was performed using SPSS24 software. The normal distribution of the data was checked by Kolmogorov-Smirnov test. All data were presented as mean ± standard deviation. The independent Sample t-test was used for the inter-group comparisons, and the repeated measure for ANOVA and Bonferroni post hoc test were used for the intra-group comparisons. The significance level of the data was considered as $p < 0.05$.

RESULTS

Evaluation of immune system activity, Intrinsic immunity, Serum bactericide

The evaluation of the serum bactericidal activity against Escherichia coli showed its high level on the days 7 and 14 after the surgery in OHE group compared to that in OVE group ($p < 0.05$). Chart 1 shows the serum bactericidal activity changes against Escherichia coli.

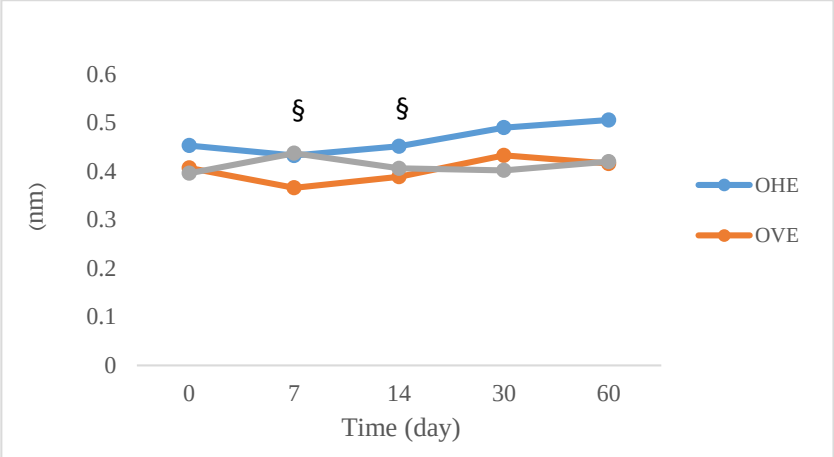

Chart 1: Changes in serum bactericidal activity against E. coli in OHE, OVE and Control groups
(OHE Group: ovariohysterctomy group, OVE Group: ovariectomy group, Control Unit: only Surgery)
§ significant differences in OHE group compared to OVE group

The evaluation of changes in serum bactericidal activity against Staphylococcus aureus did not show any significant differences among the three groups ($p > 0.050$. Chart 2 shows the serum bactericidal activity against Staphylococcus aureus.

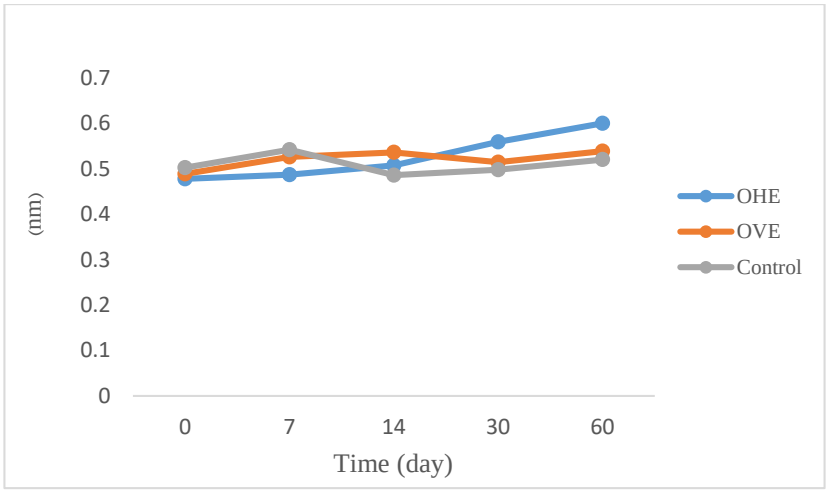

Chart 2- Changes in serum bactericidal activity against Staphylococcus aureus among three groups of HOE, OVE, and Control

(OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: only surgery).

Evaluation of complement system activity

In the secondary pathway, an increase was seen in the complement activity by the day 7, then, the reduction was seen in this activity in the test groups.

In the classic pathway, the complement activity remained fixed by the day 7 in the test groups, but it was reduced in the control group. The highest reduction in this activity was observed between days 14 and 30 after the surgery. Both secondary and classic pathways showed reduction in complement activity after the day 7. The classic pathway and to some extent, the secondary pathway showed a normal activity after the day 30.

Secondary pathway

The evaluation of the complement system secondary pathway activity showed that its values were lower in the OHE and OVE groups compared to those in the control group on days 14 and 30 after the surgery ($p < 0.05$). Moreover, the level of complement activity on day 60 in the control group was significantly higher than that in OHE group ($p < 0.05$). Chart 3 shows the changes in the activity of the complement system's secondary pathway.

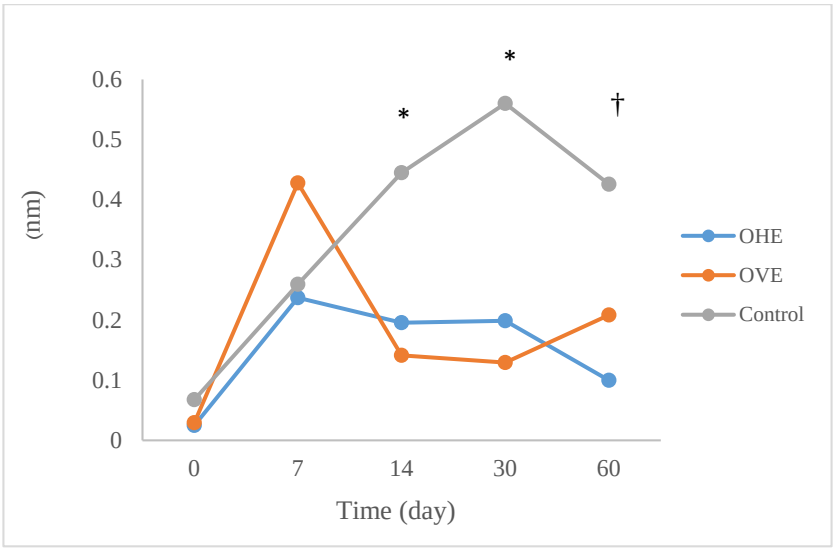

Chart 3: Changes in the activity of the complement system's secondary pathway in three groups of OHE, OVE and Control

(OHE Group: ovariohysterctomy group, OVE Group: ovariectomy group, Control group: only surgery).

*significant difference between OHE and OVE groups

†Significant difference between the control group and OVE group

Evaluation of the activity in classic pathway of the complement system

The evaluation of the classic pathway of the complement system showed that its values were in the OHE and OVE groups compared to those in the control group on day 14 after the surgery ($p < 0.05$). Chart 4 shows the changes in the activity of classic pathways of the complement system.

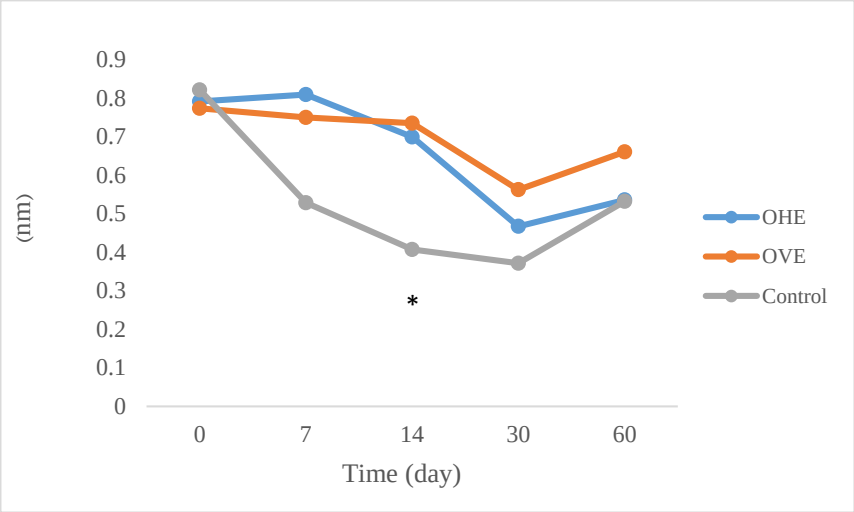

Chart 4: Changes in the activity of the complement system's classic pathway in three groups of OHE, OVE and Control

(OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: only surgery).

*significant difference between OHE and OVE groups

Lysozyme enzyme activity

The evaluation of lysozyme activity showed a significant increase in its value at day 7 after the surgery in the OHE and OVE groups compared to than in the control group ($p < 0.05$). Chart 5 shows the changes in lysozyme activity. Both Ovariectomy and Ovariohysterctomy groups remained unchanged until the day 7, but Lysozyme enzyme activity was reduced sharply until the day 14 in Ovariectomy group, and its value was normalized in Ovariohysterctomy group until the day 30.

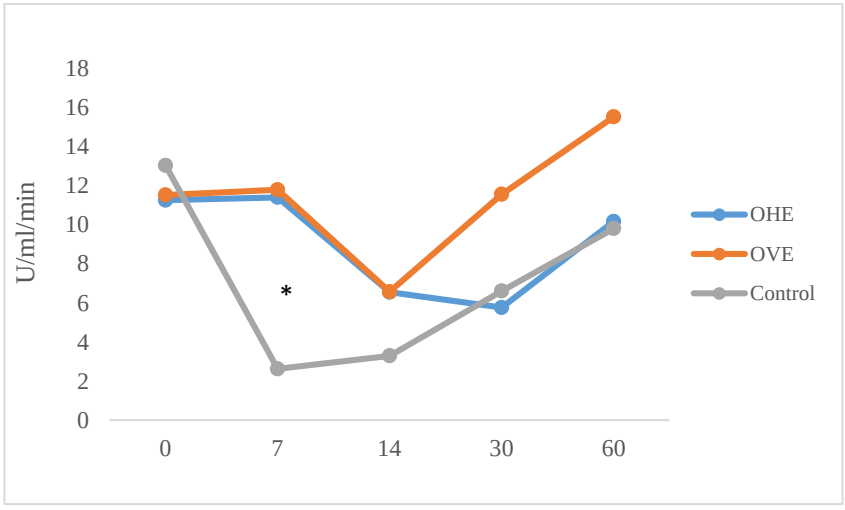

Chart 5: changes in activity of Lysozyme enzyme in three groups of OHE, OVE and Control

(OHE Group: ovariohysterctomy group, OVE Group: ovariectomy group, Control group: only surgery).

*significant difference between OHE and OVE groups

Myeloperoxidase enzyme activity

The evaluation of myeloperoxidase activity showed that its value was higher in the control group compared to that in the OHE and OVE groups at the day 7 after the surgery ($p < 0.05$). Chart 6 shows the changes in the level

of myeloperoxidase activity. Myeloperoxidase was increased in all 3 groups until the day 7. Then, its value was reduced in ovariectomy, and its value was normalized in ovariohysterctomy up to 60 days. A reduction of 7 to 14 was seen in them.

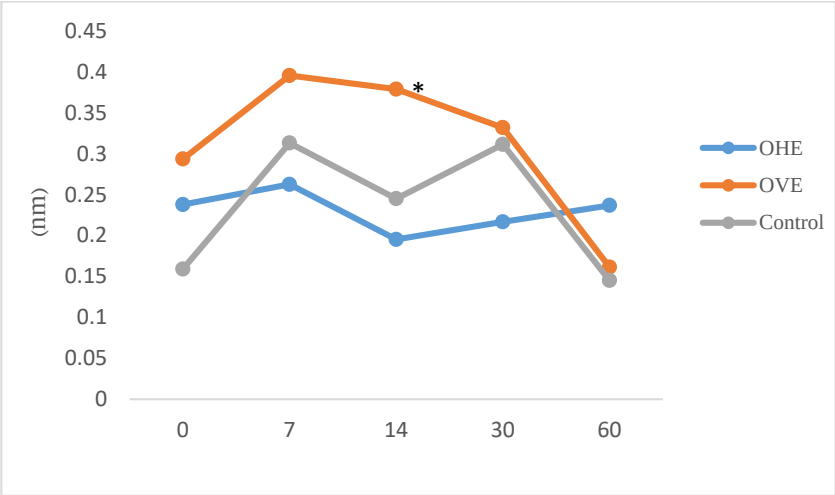

Chart 6: Changes in the activity of Myeloperoxidase enzyme in three groups of OHE, OVE and Control (OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: only surgery).
*significant difference between OHE and OVE groups

Evaluation of antitrypsin activity

The evaluation of antitrypsin activity showed a significant reduction in OHE group on day 7 after the surgery compared to that in the control and OVE groups ($p < 0.05$). Figure 7 shows the changes in the activity of antitrypsin. The antitrypsin remained unchanged in the control group, reduced sharply in ovariohysterctomy group up to the day 7, and it was normalized up to the day 30. Its value increased up to the day 7 , and it was normalized up to the day 60 in the ovariectomy group (Chart 7).

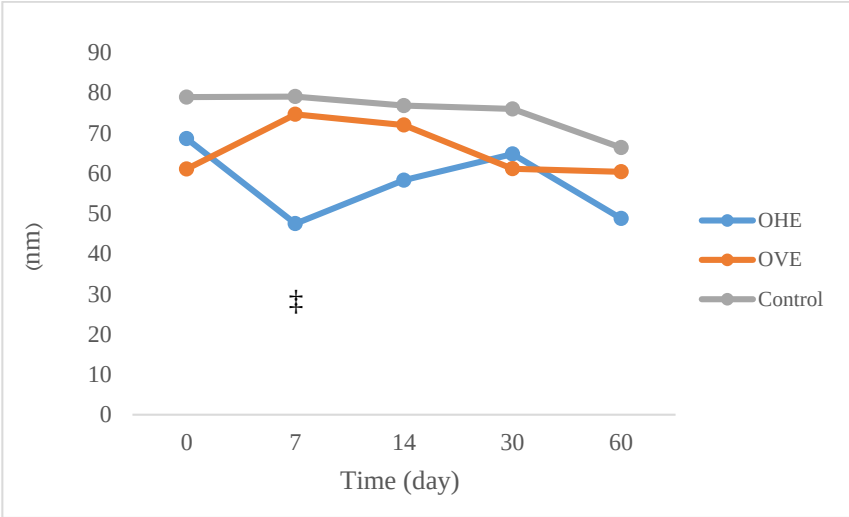

Chart 7: Changes in the activity of antitrypsin in three groups of OHE, OVE and Control (OHE Group: ovariohysterctomy group, OVE Group: ovariectomy group, Control group: only surgery).
‡A significant difference between OVE and control groups

Humoral immunity

Total serum immunoglobulin level

Comparison of the total serum immunoglobulin levels showed its higher values on day 7 after the surgery in the control group than that in the other two groups ($p < 0.05$). Chart 8 shows the changes in the total serum immunoglobulin levels.

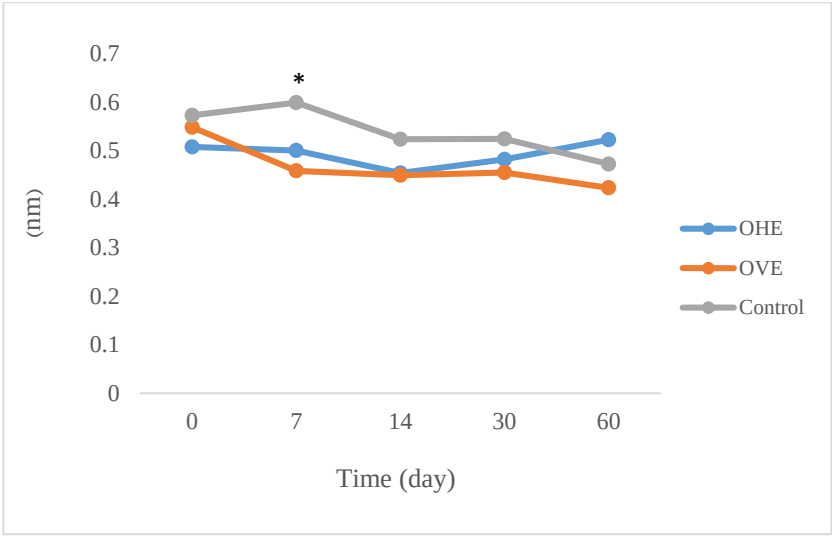

Chart 8: Changes in the total serum immunoglobulin level in three groups of OHE, OVE and Control (OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: only surgery).
* A significant difference between OVE and OHE groups

Micro-agglutination

Anti-salmonella antibody level

The comparison of anti-Salmonella anti-body level did not show any significant differences between the two groups and within each group ($p < 0.05$). Chart 9 shows the changes of anti-Salmonella antibody levels. Anti-Salmonella increased in ovariectomy and control groups up to the day 7, and this increase was higher in the control group than that in Ovariectomy group. Anti-Salmonella level became zero up to the day 30. In the ovariohysterctomy, there was no response to Salmonella injection.

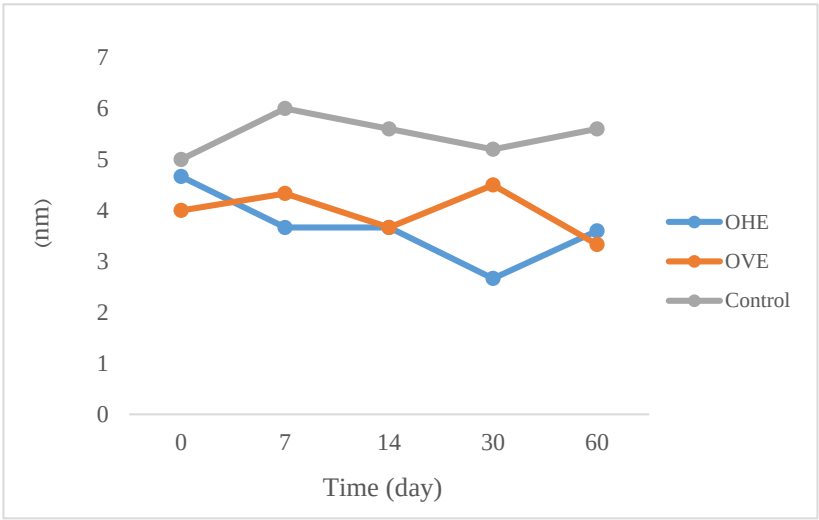

Chart 9: Changes in anti-salmonella antibody level in three groups of OHE, OVE and Control (OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: Only surgery).

Sheep anti-red blood cells antibody level

The comparison of the sheep anti-red blood cells antibody levels showed that its value was higher in the control group on day 14 after the surgery than that in OHE and OVE groups ($p < 0.05$). Chart 10 shows the changes in the level of sheep anti-red blood cells antibody. The anti-red blood cell level did not change until the day 7. During the days between 7 and 14, its level was more in the control group than that in the other two groups. Then, its level was reduced up to the day 60. After day 60, its level reached zero. This response was similar in both Ovariohysterctomy and Ovariectomy groups.

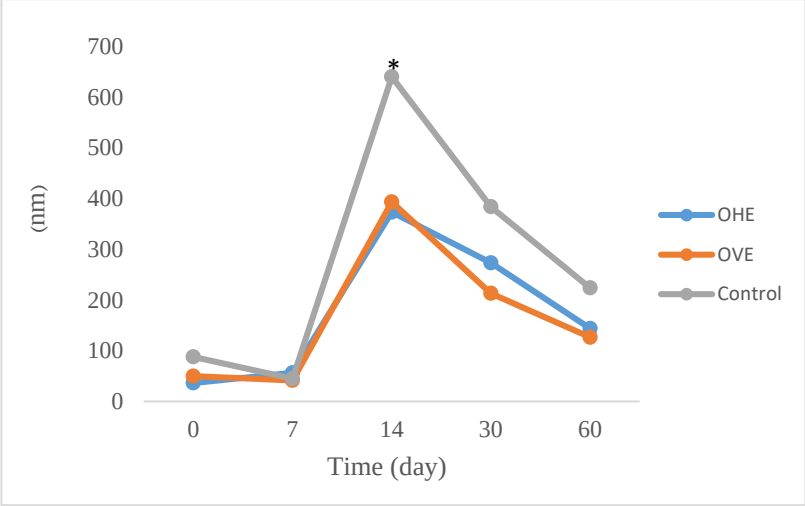

Chart 10: Changes in sheep anti-red blood cells antibody level in three groups of OHE, OVE and Control (OHE Group: Ovariohysterctomy group, OVE Group: Ovariectomy group, Control group: only surgery).
* A significant difference between OVE and OHE groups

DISCUSSION

The stimulation of the cellular and humoral immune responses following the infection and vaccination in women is more than men. Moreover, the rate of most autoimmune diseases in women is more than that of men, which its severity decreases during pregnancy. The reason for this difference is the significant effect of estrogen and progesterone hormones on inherent and acquired immune responses [15]. Various observations have proved that sex hormones can affect the immune system. For example, it can be referred to the increased autoimmune diseases in the female population, thymus hypertrophy after ovariectomy, or the suppression of cellular and mitochondrial responses during pregnancy [16]. Ovaries produce three groups of sex steroids, including estrogen, progesterone and androgen.

Previous studies have emphasized on the effects of sex hormones on specific immune responses, while the current evidence was based on the role of estrogens and testosterone in the production of antibodies which reflected an increase in the production of antibodies through estrogen and its reduction through progesterone which can be due to the effect of non-specific immune responses in various reproductive processes such as ovulation. In fact, ovaries can influence the nonspecific immune responses by affecting the number and function of monocytes, granulocytes, and NK cells [17].

Estrogen affected the immune system by affecting estrogen receptors or non-receptor-dependent mechanisms. It left different immune effects on different tissues. It sometimes increased the immunity, and sometimes reduced the immunity. The prevention of the expression of TNFα gene has been observed in T-helper lymphocytes, macrophages and dendritic cells. NFKB stimulation by estrogen led to the expression of inflammatory cytokines such as IL1, IL10 and IFNy [18]. Both pre-inflammatory and anti-inflammatory effects were observed in different organs under the effect of estradiol administration. The increased myeloperoxidase, interleukin-8 and TNFα, and respiratory burst have been observed in the estradiol administration. An increase in the derivatives of free oxygen radicals caused the imbalance of protease-antiproterase [19]. Ovariectomy in rats led to an increase in prostaglandins and inflammatory responses 42 days after the surgery [20].

In this study, the bactericidal pattern of the control group was similar to that of Escherichia coli and Staphylococcus aureus bacteria. The ovariohysterctomy group showed increasing pattern since day 7, but this trend was decreased for ovariectomy group. Moreover, on days 7 and 14, the bactericidal activity against the Escherichia coli bacterium in ovariohysterctomy group was significantly more than that of ovariectomy group. However, this difference was not observed compared to Staphylococcus aureus. The reduced bactericidal activity in the ovariectomy group compared to Gram-negative bacteria with a relatively better status of this group was not consistent with the intrinsic immunity factors. The differences in the activity of the other intrinsic immunity components affected the gram-negative bacteria, including peptides with antibacterial activity such as defensin, clectin, and pentaxin which might be effective in this regard.

Moreover, the differences in the activity of proteins carrying heavy metal carriers such as ceruloplasmin, hemopexin and the differences in the free salts required for the bacterial growth can be considered as the other potential reasons in this regard. In previous studies, despite the increased expression of some macrophage-dependent genes, such as CD68 and CD11b, in rats which underwent ovariectomy, the reduced expression of genes such as aromatase and semaphorin 3A was observed [21]. In this regard, the development of an experimental chlamydial infection in the uterus of rats and the administration of estrogen, progesterone, or a combination of them showed that progesterone increased and estrogen reduced the sensitivity of the chlamydial infection in uterine of the rats [22]. In investigating the complement activity, the activity of the secondary pathway increased, and the activity of the classic pathway was reduced up to day 30 in the control group, and a small change was seen in the activities in the test groups.

The role of the inflammatory hormones and the elimination of the effect of sex hormones in increasing factors affecting the classic pathway (C1, C4 and C2) can be considered as reasons for a higher level of these components in the ovariohysterctomy and ovariectomy groups. The highest activity in secondary pathway of the complement system was seen in the day 7 after the surgery in the ovariectomy group. In accordance with this result, an increase in the expression of the genes involved in the secondary pathway of the complement system, including C3, Factor B and properdin was reported in rats which underwent ovariectomy [21]. Lysozyme reduction in the control group in the studied time period can be attributed to the surgery-induced inflammation, and the higher levels of this enzyme in the ovariohysterctomy and ovariectomy groups can be attributed to the elimination of the inhibitory role of sex hormones in the production of this enzyme by blood leukocytes. The proximity of serum antitrypsin values in the ovariectomy group and the control group can be attributed to the better health of these animals compared to the hysterectomy group. The lowest change in myeloperoxidase activity was observed in the ovariohysterctomy group, and the highest change was observed in the ovariectomy group. The lower level of estradiol H2O2 reduced the activity of myeloperoxidase, but in the cases where H2O2 level was high, this trend was reversed [23]. In other studies, the administration of high levels of estradiol cypionate in sterilized male caws had no significant effect on the absolute and relative number of leukocytes, function of neutrophils, blastogenesis of lymphocytes, or blood cortisol.

In addition, the administration of high doses of progesterone in these cows significantly increased the migration of neutrophils, and reduced the activity of the myeloperoxidase -dependent antimicrobial system, but it had no effect on blaspogenic response to mitogens or the ability of polymorphonuclear leukocytes to degrade Staphylococcus aureus; Superoxide production reduced the NBT salt, and antibody-dependent cell cytotoxic activity [24]. In addition, ovariectomy in rats reduced the expression of interleukin -12, TNFα and TLR2 genes in the brain phagocytic cells [25]. The reduction of some of the intrinsic immunity factors in the current study could be attributed to the reduction in the production of cytokines and intrinsic immune receptors. Comparing the total serum immunoglobulin levels showed that its value was higher on day 7 after the surgery in the control group. Its level showed a slight reduction in ovariectomy in control groups from day 30 to 60.

A slight increase in the total antibody acid level of the ovariohysterctomy group was observed from day 14 to day 60, indicating the lack of the inhibitory effect of sex hormones and lymphocytes regulating the immune system activity. Increasing corticosteroid hormones caused by stress and inflammation could be attributed to the complete suppression of the response to the administration of killed bacterium salmonella typhimurium in the ovariohysterctomy and the slight suppression of the ovariectomy group. Given the role of T lymphocytes in humoral immune response to red blood cells injection, the same repression in both groups could be attributed to the reduction of anti-SRBC antibody level compared to the control group. In the research conducted by Kuhn et al (1991), despite the reduction in anti-RBC antibody in rats undergoing ovariohysterctomy and ovariectomy, the response was not significant [26]. The effects of estrogen influenced the maturation of lymphocytes B and T [27, 28] and reduced the production of lymphocytes B by reducing the interleukin -7 production by the bone marrow stromal cells [28].

Estrogen increased the cells with CD4 + and CD8- phenotypes, and the ovariectomy shifted this phenotype to CD4- and CD8 + [29, 30]. Estrogen also increased TH2 responses, and increased the level of interleukin 4, 5, and 10. The reduction in the lymphocyte T-dependent humoral immunity antibody level in response to sheep RBC administration might be attributed to the reduction in the level of T-helper lymphocytes in the groups undergoing ovariohysterctomy and ovariectomy. Estrogen has been involved in many female reproductive activities such as fertility and pregnancy [31]. However, the specific estrogen function in the immunity activities

has not been identified [32] and both positive and negative role of estrogen in immunity functions have been reported [33-35]. It has been shown that estrogen had a repressive effect on the immunity function [32]. This hormone also increased the humoral immunity function and cytokine levels slightly [32]. For example, estrogen stimulated B cells and increased the antibody production [36, 37]. Progesterone hormone receptors were expressed on lymphocytes during pregnancy [17, 38]. Androgen receptors were found on B and T lymphocytes [39, 40]. Androgens increased the response of TH1, and thus, increased interleukin 2 and increased the activation of TCD8 + cells [17, 27].

Progesterone increased the expression of sticky molecules on leukocytes, and caused a change in the level of circulating cells and affected the cell activation [41]. For example, it affected the hormonal levels in different phases of the estrous cycle of neutrophils. During the menstrual cycle, the rate of neutrophil decreased, in the follicular phase, and the endometrium of the neutrophils increased, and after the ovulation, this increase continued [42, 43]. In a review study to compare the surgeries performed in female dogs between 1996 and 2004, Goethem et al., (2006) stated that ovariohysterctomy, compared to ovariectomy, was associated with more complications and time [44].

The researchers reported that the possible complications of ovariohysterctomy have been intra-abdominal bleeding, ligation, ovarian survival syndrome, and the complications caused by the remains of uterine and sinus tract formation. The rate of short-term complications in both surgical procedures was similar. In this study, the ovariectomy method was introduced as a selective sterilization method for dogs. DeTora and McCarti (2011) also stated that ovariohysterctomy had no superiority to ovariectomy in dogs and cats, and ovariectomy can reduce the complications of ovariohysterctomy [1]. Additionally, Peeters and Kirpensteijn (2011) examined the short-term complications of ovariohysterctomy and ovariectomy on 40 dogs [45]. No difference was found between the two procedures in terms of pain during the surgery and level of the ulcer recovery [45].

In this study, surgery led to the changes in inherent immunity activities. In the control group, an increase in the activity of myeloperoxidase and serum bactericidal activity, a reduction in lysozyme activity and complement, and the lack of change antitrypsin activity were observed up to day 7. These changes could be due to the surgery inflammation, the use of some immunity factors, the effect of corticosteroid hormones, and the analgesic and sedative and anti-pain drugs, as all the activities were normalized and returned to their initial levels over time since day 7 to day 60. However, the level of activity of complement secondary pathway remained more than that of zero time, and the level of activity of classic pathway remained less than that of zero time, and the activity of complement and myeloperoxidase moved towards the normal level since day 30. The ovariohysterctomy and ovariectomy surgeries, in addition to the complications mentioned in the control group, led to the elimination of sex hormones. This case led to the difference in the pattern of changes in the test groups. Except for antitrypsin, the changes in the other factors in the test groups, compared to those in the control group were less. A significant difference in the activity of complement, myeloperoxidase and lysozyme, compared to the control group, was one of the changes. The total count of immunoglobulin in the blood and salmonella and anti-RBC antigens in the test groups was reduced in comparison with the control on day 7. In general, the acquired weaker immune response and the deviation of the inherent immune responses were some of the cases observed in this study. No difference was observed in the effects of ovariohysterctomy and ovariectomy on nonspecific immune responses.

REFERENCES

1. DeTora, M., McCarthy, R.J., 2011. Ovariohysterectomy versus ovariectomy for elective sterilization of female dogs and cats: is removal of the uterus necessary? Journal of the American Veterinary Medical Association 239, 1409-1412.
2. Kustritz, M.V.R., 2007. Determining the optimal age for gonadectomy of dogs and cats. Journal of the American Veterinary Medical Association 231, 1665-1675.
3. Root Kustritz, M., 2012. Effects of surgical sterilization on canine and feline health and on society. Reproduction in domestic animals 47, 214-222.
4. Hart, B.L., Hart, L.A., Thigpen, A.P., Willits, N.H., 2014. Long-term health effects of neutering dogs: comparison of Labrador Retrievers with Golden Retrievers. PloS one 9, e102241.
5. Kustritz, M.V.R., 2002. Early spay-neuter: clinical considerations. Clinical techniques in small animal practice 17, 124-128.

6. Shelton, G.D., Schule, A., Kass, P.H., 1997. Risk factors for acquired myasthenia gravis in dogs: 1,154 cases (1991-1995). Journal of the American Veterinary Medical Association 211, 1428-1431.
7. Shelton, G., 1998. Myasthenia gravis: lessons from the past 10 years. Journal of small animal practice 39, 368-372.
8. Jacobs, R., Murtaugh, R., Crocker, D., 1984. Use of a microtiter Coombs' test for study of age, gender, and breed distributions in immunohemolytic anemia of the dog. Journal of the American Veterinary Medical Association 185, 66-69.
9. Shaker Ali, S. Mahassni, S.H. & Alnefaie, R.M. (2018). The Effects of Hypervitaminosis D in Rats on Histology and Weights of Some Immune System Organs and Organs Prone to Calcification. International Journal of Pharmaceutical and Phytopharmacological Research, 8(6), pp.59-71.
10. Mahassni, S.H. & Bashanfar, N.O. (2019). High Levels of Inflammatory Adipokines and C-reactive protein, and Minimal Changes in Immune Cells in Overweight and Obese Saudi Female University Students. International Journal of Pharmaceutical Research & Allied Sciences, 8(1):171-183.
11. Fossum, T.W.2013. Small animal surgery. 4th ed. St. Louis, Mo. : Elsevier Mosby
12. Shugar, D., 1952. The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. Biochimica et biophysica acta 8, 302-309.
13. Sahoo, P., Kumari, J., Mishra, B., 2005. Non-specific immune responses in juveniles of Indian major carps. Journal of Applied Ichthyology 21, 151-155.
14. Sedlinská, M., Krejčí, J., Vyskočil, M., 2005. Evaluation of field methods for determining immunoglobulin in sucking foals. Acta Veterinaria Brno 74, 51-58.
15. Beagley, K.W., Gockel, C.M., 2003. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunology & Medical Microbiology 38, 13-22.
16. Bhalla, A., 1989. Hormones and the immune response. Annals of the rheumatic diseases 48, 1.
17. Bouman, A., Heineman, M.J., Faas, M.M., 2005. Sex hormones and the immune response in humans. Human reproduction update 11, 411-423.
18. Khan, D., Cowan, C., Ahmed, S.A., 2012. Estrogen and signaling in the cells of immune system. Advances in Neuroimmune Biology 3, 73-93.
19. Abid, S., Xie, S., Bose, M., Shaul, P.W., Terada, L.S., Brody, S.L., Thomas, P.J., Katzenellenbogen, J.A., Kim, S.H., Greenberg, D.E., 2017. 17β-estradiol dysregulates innate immune responses to pseudomonas aeruginosa respiratory infection and is modulated by estrogen receptor antagonism. Infection and immunity, IAI. 00422-00417.
20. Ibáñez, L., Alcaraz, M.J., Maicas, N., Guede, D., Caeiro, J.R., Koenders, M.I., van den Berg, W.B., Ferrándiz, M.L., 2011. Up-regulation of the inflammatory response by ovariectomy in collagen-induced arthritis. effects of tin protoporphyrin IX. Inflammation 34, 585-596.
21. Sarvari, M., Kalló, I., Hrabovszky, E., Solymosi, N., Liposits, Z., 2014. Ovariectomy and subsequent treatment with estrogen receptor agonists tune the innate immune system of the hippocampus in middle-aged female rats. PloS one 9, e88540.
22. Kaushic, C., Zhou, F., Murdin, A.D., Wira, C.R., 2000. Effects of estradiol and progesterone on susceptibility and early immune responses to Chlamydia trachomatis infection in the female reproductive tract. Infection and immunity 68, 4207-4216.
23. Klebanoff, S.J. 1980. Myeloperoxidase-mediated cytotoxic systems, In: Biochemistry and Metabolism. Springer, 279-308.
24. Roth, J.A., Kaeberle, M.L., Hsu, W.H., 1982. Effect of estradiol and progesterone on lymphocyte and neutrophil functions in steers. Infection and immunity 35, 997-1002.
25. Soucy, G., Boivin, G., Labrie, F., Rivest, S., 2005. Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. The Journal of Immunology 174, 6391-6398.
26. Kuhn, G., Waldherr, R., Maser-Gluth, C., Hardegg, W., Vecsei, P., 1991. Long-term effects of ovariectomy on pituitary-adrenal axis and specific antibody response in rats. Research in experimental medicine 191, 327-337.
27. Ackerman, L.S., 2006. Sex hormones and the genesis of autoimmunity. Archives of Dermatology 142, 371-376.

28. Hill, L., Jeganathan, V., Chinnasamy, P., Grimaldi, C., Diamond, B., 2011. Differential roles of estrogen receptors α and β in control of B-cell maturation and selection. Molecular Medicine 17, 211.
29. Shames, R.S., 2002. Gender differences in the development and function of the immune system. Journal of Adolescent Health 30, 59-70.
30. Seli, E., Kayisli, U.A., Selam, B., Seli, M., Arici, A., 2002. Estradiol suppresses vascular monocyte chemotactic protein-1 expression during early atherogenesis. American journal of obstetrics and gynecology 187, 1544-1549.
31. Ellison, P.T., 2003. Energetics and reproductive effort. American Journal of Human Biology 15, 342-351.
32. Foo, Y.Z., Nakagawa, S., Rhodes, G., Simmons, L.W., 2017. The effects of sex hormones on immune function: a meta-analysis. Biological Reviews 92, 551-571.
33. Adori, M., Kiss, E., Barad, Z., Barabás, K., Kiszely, E., Schneider, A., Sziksz, E., Ábrahám, I.M., Matkó, J., Sármay, G., 2010. Estrogen augments the T cell-dependent but not the T-independent immune response. Cellular and molecular life sciences 67, 1661-1674.
34. Douin-Echinard, V., Calippe, B., Billon-Galès, A., Fontaine, C., Lenfant, F., Trémollières, F., Bayard, F., Guéry, J.C., Arnal, J.F., Gourdy, P., 2011. Estradiol administration controls eosinophilia through estrogen receptor-α activation during acute peritoneal inflammation. Journal of leukocyte biology 90, 145-154.
35. Zhou, W., Song, Y., Xu, H., Zhou, K., Zhang, W., Chen, J., Qin, M., Yi, H., Gustafsson, J.-A., Yang, H., 2011. In nonfunctional pituitary adenomas, estrogen receptors and slug contribute to development of invasiveness. The Journal of Clinical Endocrinology & Metabolism 96, E1237-E1245.
36. Grimaldi, C.M., Hill, L., Xu, X., Peeva, E., Diamond, B., 2005. Hormonal modulation of B cell development and repertoire selection. Molecular immunology 42, 811-820.
37. Orbach, H., Shoenfeld, Y., 2007. Hyperprolactinemia and autoimmune diseases. Autoimmunity reviews 6, 537-542.
38. Sader, M.A., McGrath, K.C., Hill, M.D., Bradstock, K.F., Jimenez, M., Handelsman, D.J., Celermajer, D.S., Death, A.K., 2005. Androgen receptor gene expression in leucocytes is hormonally regulated: implications for gender differences in disease pathogenesis. Clinical endocrinology 62, 56-63.
39. Altuwaijri, S., Chuang, K.-H., Lai, K.-P., Lai, J.-J., Lin, H.-Y., Young, F.M., Bottaro, A., Tsai, M.-Y., Zeng, W.-P., Chang, H.-C., 2009. Susceptibility to autoimmunity and B cell resistance to apoptosis in mice lacking androgen receptor in B cells. Molecular Endocrinology 23, 444-453.
40. Benten, W.P.M., Lieberherr, M., Stamm, O., Wrehlke, C., Guo, Z., Wunderlich, F., 1999. Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Molecular Biology of the Cell 10, 3113-3123.
41. Chernyshov, V., Vodianyk, M., Hrekova, S., 2002. Effect of female steroid hormones on expression of adhesion molecules by peripheral blood leukocytes. Fiziolohichnyi zhurnal (Kiev, Ukraine: 1994) 48, 46-53.
42. England, J., Bain, B.J., 1976. Total and differential leucocyte count. British journal of haematology 33, 1-7.
43. Jiemtaweeboon, S., Shirasuna, K., Nitta, A., Kobayashi, A., Schuberth, H.-J., Shimizu, T., Miyamoto, A., 2011. Evidence that polymorphonuclear neutrophils infiltrate into the developing corpus luteum and promote angiogenesis with interleukin-8 in the cow. Reproductive Biology and Endocrinology 9, 79.
44. Van Goethem, B., SCHAEFERS-OKKENS, A., Kirpensteijn, J., 2006. Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. Veterinary Surgery 35, 136-143.
45. Peeters, M.E., Kirpensteijn, J., 2011. Comparison of surgical variables and short-term postoperative complications in healthy dogs undergoing ovariohysterectomy or ovariectomy. Journal of the American Veterinary Medical Association 238, 189-194.