https://doi.org/10.52973/rcfcv-e33290 Revista Científica, FCV-LUZ / Vol. XXXIII, rcfcv-e33290

Investigation of changes in Hematological parameters and levels of Oxidative stress factors in castrated Cats

Investigación de cambios en parámetros hematológicos y niveles de factores de estrés oxidativo en gatos castrados

Eren Polat[1]*, Tutku Can Acisu[2], Mustafa Sönmez[2], Emine Ünsaldi[1], Önder Otlu[3], Murat Tanrisever[1], Aslıhan Çakır[2], Yasin Baykalir[4]

[1]*Firat University, Faculty of Veterinary Medicine, Department of Veterinary Surgery. Elazig, Turkey.*
[2]*Firat University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination. Elazig, Turkey.*
[3]*Turgut Ozal University, Faculty of Medicine, Department of Medical Biochemistry. Malatya, Turkey.*
[4]*Balikesir University, Faculty of Veterinary Medicine, Department of Veterinary Biostatistics. Balikesir, Turkey.*
**Corresponding author: erenpolat@firat.edu.tr*

ABSTRACT

This study was carried out to determine the effect of castration procedure on hemogram and leukogram values and the levels of oxidative stress factors in serum tissue in cats. Preoperative and postoperative 10th day hemogram/leukogram values and total oxidant/antioxidant capacities of 19 castrated cats were evaluated in the study. When the hemogram and leukogram values were examined, it was determined that there was a statistically significant increase in the amount of eosinophils ($P<0.05$). Although the increase in white blood cells (WBC), neutrophil and lymphocyte levels and the decrease in red blood cells (RBC) and hematocrit (HCT) levels were remarkable, it was not found to be statistically significant ($P>0.05$). When the levels of oxidative stress factors in the serum tissues of the cats were examined, it was determined that there was a decrease in the total antioxidant status (TAS) and an increase in the total oxidant status (TOS), but the difference in the results was not statistically significant ($P>0.05$). In conclusion, in this study, it was determined that the castration procedure in cats did not cause a significant difference in hemogram and leukogram parameters and the level of oxidative stress factors in serum tissue, except for the amount of eosinophils.

Key words: Castration; cat; eosinophil; hemogram; oxidative stress

RESUMEN

Este estudio se llevó a cabo para determinar el efecto del procedimiento de castración sobre los valores de hemograma y leucograma y los niveles de factores de estrés oxidativo en el tejido sérico de gatos. En el estudio se evaluaron los valores de hemograma/leucograma preoperatorios y posoperatorios al décimo día y las capacidades oxidantes/antioxidantes totales 19 gatos castrados. Cuando se examinaron los valores de hemograma y leucograma en el estudio, se determinó que hubo un aumento estadísticamente significativo en la cantidad de eosinófilos ($P<0,05$). Aunque el aumento en los niveles de glóbulos blancos (WBC), neutrófilos y linfocitos y la disminución en los niveles de glóbulos rojos (RBC) y hematocrito (HCT) fueron notables, no se encontró que fueran estadísticamente significativos ($P>0,05$). Cuando se examinaron los niveles de factores de estrés oxidativo en los tejidos séricos de los gatos, se determinó que hubo una disminución en el estado antioxidante total (TAS) y un aumento en el estado oxidante total (TOS), pero la diferencia en resultados no fue estadísticamente significativa ($P<0,05$). En conclusión, en este estudio se determinó que el procedimiento de castración en gatos no provocó una diferencia significativa en los parámetros del hemograma, leucograma y el nivel de factores de estrés oxidativo en el tejido sérico, excepto la cantidad de eosinófilos.

Palabras clave: Castración; eosinófilos; estrés oxidativo; gato; hemograma

Received: 12/07/2023 Accepted: 25/08/2023 Published: 25/09/2023

INTRODUCTION

Castration is the process of eliminating the source of hormones that create sex–related physical and behavioral characteristics in order to prevent reproduction in male cats (*Felis catus*) and dogs (*Canis lupus familiaris*). Surgical removal of testicles is the most widely used castration technique in all mammalian species [1, 2]. In many Countries (such as USA, England), castration processes are used to prevent the uncontrolled population growth of cats and dogs. However, in some Countries (such as Germany, Norway, Sweden), castration is seen as an unethical practice [1, 3].

It has been stated by some international organizations (American Veterinary Medical Association, American Animal Hospital Association, British Small Animal Veterinary Association) that castration in the prepubertal period is acceptable. In some studies [1, 4], it has been stated that castrations performed at an early age in cats and dogs have no or very few side effects. However, many veterinarians oppose castration in the prepubertal period due to the orthopedic and oncological risks that may occur in later ages [1, 5]. In addition, it also causes problems such as the narrowing of the urethra, which prevents the urinary catheter from passing, and the increase in obesity.

Today, there are discussions about the advantages and disadvantages of the castration process. With the castration process, the excessive increase in cat and dog populations are prevented, the risk of formation of some genital system diseases (some prostate pathologies, some testicular tumors) and the control of unwanted behaviors (aggression, urinating in inappropriate places) are provided [1, 2]. Again, some studies report that the life span of castrated cats and dogs is prolonged [6, 7]. Castration has both advantages and disadvantages. Some studies have found an increased incidence of osteosarcoma and prostate tumors in castrated cats [1, 8, 9, 10]. Again, some studies report an increased risk of obesity [11, 12, 13], some orthopedic problems [14, 15], and diabetes mellitus (especially Burmese breed) [1, 16] in cats after castration.

It is known that the castration process has an effect on some hematological parameters and the levels of oxidative stress factors [2, 17, 18]. Aengwanich *et al*. [2] reported that there were significant changes in the neutrophil/lymphocyte ratio in dogs after castration (surgical method). Surgical operations are one of the important stress factors for living beings. The deterioration of the oxidant/antioxidant balance in the body in favor of oxidants under the influence of any stress factor is called oxidative stress. Oxidative stress causes the body's resistance to degenerative diseases to decrease. In addition to causing cardiovascular system diseases and neurodegenerative central nervous system diseases, it also causes oncological diseases by damaging the Deoxyribonucleic acid (DNA) helix [2, 19, 20, 21, 22, 23, 24].

In this study, changes in some blood parameters and oxidative stress factors in preoperative and postoperative periods were evaluated in castrated cats. Thus, by investigating the effect of castration on the formation of oxidative stress, it was aimed to evaluate the decrease or increase in body resistance against some degenerative diseases in the body. At the same time, it was determined whether the castration process would cause a change in some blood parameters, and its effect on the health status of cats was investigated.

MATERIALS AND METHODS

Ethical approval and study plan

This study was carried out in accordance with ethical principles with the approval of Fırat University Animal Experiments Local Ethics Committee (dated: 22.03.2021, numbered: 28617). The study was carried out on 19 cats brought to Fırat University Animal Hospital Surgery Clinic for castration. In the study, the changes in the levels of some blood parameters and oxidative stress factors in the preoperative and postoperative period of the cats were evaluated.

Collecting blood samples

For the study, 5 mL blood was collected from cats both in tubes with 10% Ethylenediaminetetraacetic acid (EDTA) and in tubes with vacuum gel and clot activator on the preoperative and postoperative 10th day. The cephalic vein in the forearm was used when blood samples were taken.

Anesthesia and castration procedure

Before the castration procedure, 2 mg·kg^{-1} Xylazine hydrochloride was administered intramuscularly to the cats. After 10 min, anesthesia was achieved by administering Ketamine hydrochloride at a dose of 10 mg·kg^{-1} intramuscularly [25].

After shaving and disinfection of the scrotum of the cats, the operation area was limited to the operation cover. The testicles were limited and the scrotum skin was tightened (FIG. 1A). An incision long enough for the testicles to protrude was made into the skin of the scrotum, parallel to the *raphe scroti* (FIG. 1B,1C). After the soft tissues and *tunica vaginalis* were cut, the testis was taken out and separated from all its connections (FIG. 1D). After the hemostatic forceps were placed on the part of the testicular cord towards the body, a ligature was applied by transfixation method using absorbable suture material (FIG. 1E,1F). The same procedure was performed for the second testis. In the postoperative period, Povidin–iodine solution was applied to the operation wound for 3 days (d) and parenteral antibiotics (Sülcid, 0.25 mg) were administered for 3 d. For postoperative pain management, 0.2 mg·kg^{-1} meloxicam was administered subcutaneously to all cats.

The cats that made up the material of the study were from different breeds, aged 12–36 months. The surgeons performing and assisting the operations were the same in all operations. Each of the operations lasted an average of 3–4 min. All cats included in this study were castrated during the summer months.

Measurement of hematological parameters

Hemogram and leukogram values of the cats were done with whole blood analyzer (Hasvet Mindray BC–5000 Vet branded, Turkey). In order to determine the oxidative stress factors, blood samples in tubes with vacuum gel and clot activator were centrifuged at 3000 G for 10 min.

Determination of total oxidant status (TOS) activity

TOS activity of serum tissue was studied using Total Oxidant Status kit (Rel Assay Diagnostics). 250 µL of buffer solution was added to all wells. 37 µL of standard sample was added to the standard well and 37 µL of sample was added to the sample well. The first reading was made at 530 nm. 12 µL of substrate solution was added to all wells and incubated at room temperature (20 – 25°C) for 10 min with shaking.

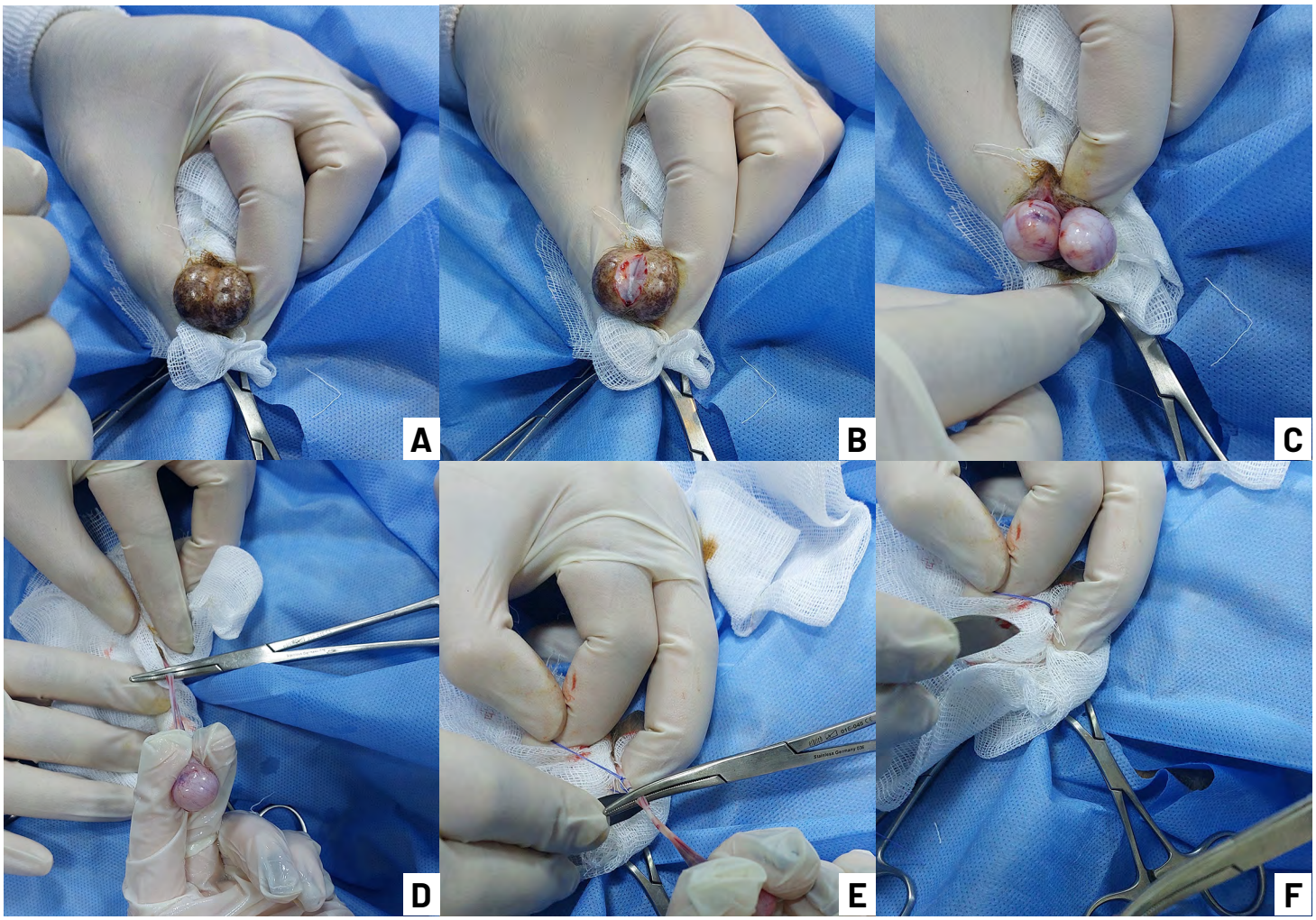

FIGURE 1. Stages of the surgical castration procedure (A–F)

At the end of the incubation, a second reading was made at 530 nm and the results were calculated [26].

Determination of total antioxidant status (TAS) activity

Serum tissue TAS activity was studied using the Total Antioxidant Status kit (Rel Assay Diagnostics). 250 µL of buffer solution was added to all wells. 15 µL of standard sample was added to the standard well and 15 µL of sample was added to the sample well. The first reading was taken at 660 nm. 37 µL of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical solution was added to all wells and incubated at room temperature (20 – 25°C) for 10 min with shaking. At the end of the incubation, a second reading was made at 660 nm and the results were calculated [26].

Determination of oxidative stress index

Oxidative Stress Index (OSI), which is an indicator of oxidative stress load, was obtained by dividing the TOS value by the TAS value. Arbitrary Unit (AU) was used as the unit of OSI.

$$OSI\,(ArbitraryUnit) = \frac{(TOS,\ \mu molH_2O_2\ eq{\cdot}L^{-1})}{(TAS,\ \mu molTrolox\ eq{\cdot}L^{-1})}$$

Statistical analysis

All data on the preoperative and postoperative 10th d were evaluated in the IBM SPSS 22 package program. The Paired-Samples *t*-test was used because the data were continuous and obtained as a result of pre- and post-operative observations obtained from the same individuals. Data are presented as mean ± standard error. Statistical significance was accepted when $P \leq 0.05$ [27].

RESULTS AND DISCUSSIONS

Hemogram and leucogram results

In the study, the results of hemogram and leukograms of 19 male cats before the castration operation and on the 10th day after the castration operation were evaluated. In the examinations, it was determined that there was only a statistically significant increase in the amount of eosinophils. The changes in the hemogram and leukogram results are presented in TABLE I and FIGS. 2, 3 and 4.

Total antioxidant–oxidant (TAS–TOS) results

In the study, TAS and TOS activity and OSI results of 19 male cats were evaluated before the castration operation and on the 10th day after the castration operation. According to the obtained results, it was determined that there was a decrease in TAS activity and an increase in TOS activity, but these results were not statistically significant. The changes in TAS and TOS activities and OSI ratio are presented in TABLE II and FIG. 5.

The advantages and disadvantages of castration, which is mostly done to prevent excessive increase in cat and dog populations and some gender–related behaviors, are frequently discussed today [1]. There are studies reporting that it has advantages such as a decrease in the incidence of some genital diseases and prolongation of life span [1, 6, 7], as well as disadvantages such as obesity and an increase in the incidence of some orthopedic diseases [11, 12, 13, 14, 15]. There are studies reporting an increased incidence of osteosarcoma and prostate tumors in castrated cats [1, 9]. It is known that oxidative stress is one of the most important causes of cardiovascular system diseases, neurodegenerative central nervous system diseases and some oncological diseases [24]. The effect of castration on oxidative stress is therefore very important. In this study, it was aimed to establish the basis for new studies on the possibility of castration to have an effect on the etiology of diseases that may occur in the future by determining the preoperative and postoperative TAS, TOS and OSI values of castrated cats.

Surgical operations are one of the most important causes of stress for living things. Oxidative stress is the deterioration of the oxidant/antioxidant balance in the body in favor of oxidants, due to the effect of any stress factor. Oxidative stress causes the body to

TABLE I
Some blood parameters before and after the operation in castrated male cats

Parameter	Preoperative	Postoperative (10th day)	*P*–value
Neutrophils (cells·μL^{-1})	4,311 ± 392 [a]	5,832 ± 1,590 [a]	0.334
Neutrophil (%)	46.671 ± 4.081 [a]	44.512 ± 4.139 [a]	0.509
Lymphocyte (cells·μL^{-1})	4,285 ± 522 [a]	4,851 ± 580 [a]	0.185
Lymphocyte (%)	45.488 ± 4.457 [a]	46.306 ± 4.009 [a]	0.798
Monocyte (cells·μL^{-1})	0.085 ± 0.015 [a]	0.119 ± 0.044 [a]	0.400
Monocyte (%)	1.000 ± 0.203 [a]	0.853 ± 0.124 [a]	0.430
Eosinophil (cells·μL^{-1})	0.657 ± 0.101 [a]	0.839 ± 0.120[b]	0.015
Eosinophil (%)	6.806 ± 0.763 [a]	8.241 ± 1.108 [a]	0.078
Basophil (cells·μL^{-1})	0.005 ± 0.002 [a]	0.016 ± 0.004 [a]	0.066
Basophil (%)	0.060 ± 0.022 [a]	0.150 ± 0.058 [a]	0.204
WBC (cells·μL^{-1})	9,288 ± 594 [a]	11,651 ± 1,549 [a]	0.154
RBC (cells×10^3·μL^{-1})	11,075 ± 589 [a]	10,838 ± 427 [a]	0.615
HCT (%)	40.553 ± 1.485 [a]	40.153 ± 1.430 [a]	0.809
HGB (g·dL^{-1})	14.706 ± 0.542 [a]	14.600 ± 0.419 [a]	0.850
MCV (fL)	37.253 ± 1.143 [a]	37.324 ± 0.961 [a]	0.921
PLT (platelet·μL^{-1})	236,590 ± 24,747 [a]	236,706 ± 28,732 [a]	0.996

WBC: White Blood Cell, RBC: Red Blood Cell, HCT: Hematocrit Value, HGB: Hemoglobin Value, MCV: Mean Corpuscular Volume, PLT: Platelet Value. [a,b]: Different letters on the same line mean statistical significance

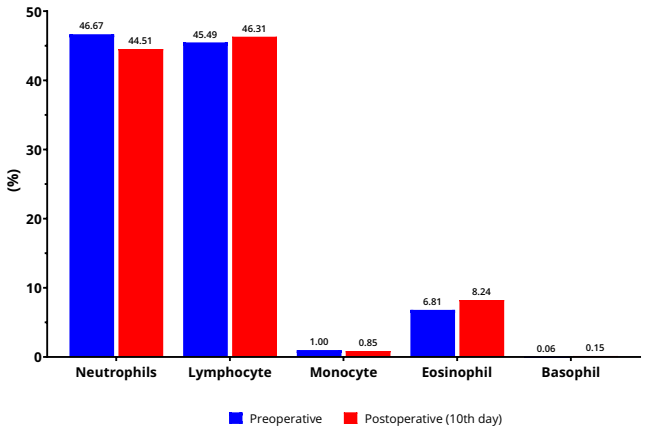

FIGURE 2. Graph of percentage of white blood cells before castration and on the 10th postoperative day

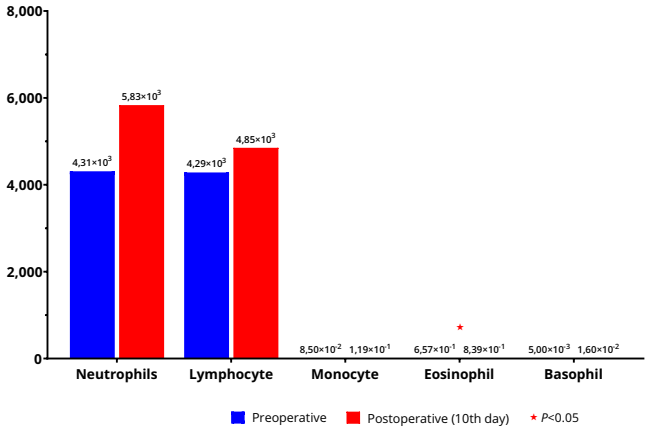

FIGURE 3. Graph of the amount of white blood cells before castration and on the 10th postoperative day

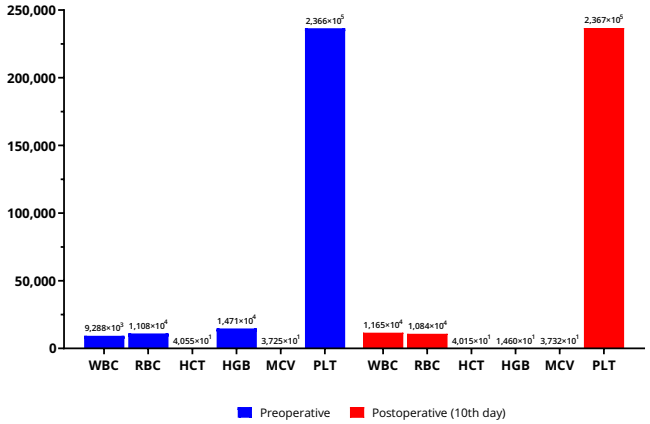

FIGURE 4. Graph of the amounts of some hemogram and leukogram parameters before castration and on the tenth postoperative day

TABLE II
Plasma total antioxidant and oxidant activity and OSI ratio in castrated male cats at the pre- and postoperative 10th day

Parameter	Preoperative	Postoperative (10th day)	*P*-value
TAS	1.738 ± 0.013[a]	1.718 ± 0.089[a]	0.656
TOS	4.294 ± 0.029[a]	4.314 ± 0.052[a]	0.710
OSI	251.390 ± 0.560[a]	251.061 ± 0.112[a]	0.583

TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index.
[a]: Different letters on the same line mean statistical significance.

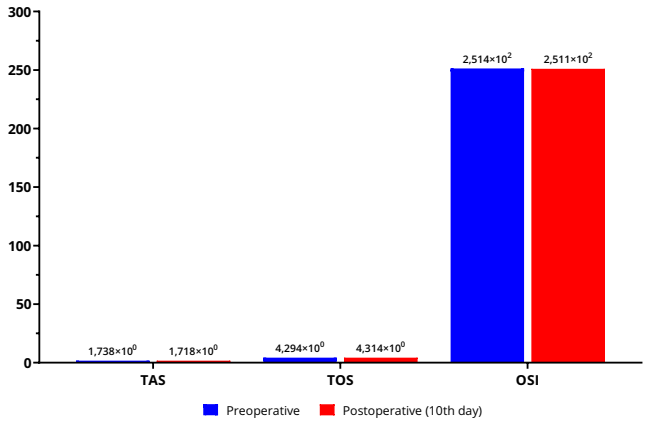

FIGURE 5. Graph of TAS, TOS levels and OSI ratio before castration and on the tenth postoperative day

decrease its resistance against degenerative diseases [22, 24]. The hypothesis that the castration process is a surgical operation and that the metabolic changes that occur after castration may have an effect on oxidative stress formed the basis of this study.

Aengwanich *et al.* [2] reported that the total antioxidant capacity of the dogs on the postoperative third day of the surgical castration decreased significantly compared to the postoperative fourteenth d ($P<0.05$). At the end of the recovery period, they reported that the total antioxidant capacity of the dogs was close to the preoperative period. In the same study, they reported that there was no statistically significant change in plasma malondialdehyde (MDA) level before, during and after castration ($P>0.05$). Mogheiseh *et al.* [28] investigated the effect of Melatonin on oxidative stress level in dogs after castration. In this study, they reported that the MDA level in dogs increased statistically significantly after castration.

In the study, it was reported that the MDA level in dogs treated with Melatonin (Castration+Melatonin group) decreased significantly compared to the dogs in the control and castration groups. They also reported that antioxidant levels such as catalase, glutathione peroxidase and superoxide dismutase were statistically significantly increased in dogs in the Melatonin-treated group. In the same study, it was reported that the MDA levels of the dogs in the castration group increased and their antioxidant levels decreased when compared to the control group. Mahallingam *et al.* [17] reported that although SOD and CAT levels increased postoperatively in dogs neutered by laparoscopic vasectomy and prescrotal surgical castration, they decreased to the preoperative level on the fifth postoperative day. In the same study, it was reported that no significant difference was found between the CAT and SOD levels of dogs in the laparoscopic vasectomy group and prescrotal surgical castration groups.

Jana and Samanta [18], evaluated oxidative stress parameters in male cats neutered by intratesticular administration of different concentrations of Calcium chloride solutions (5, 10 and 20%). In this study, it was reported that the MDA level in the testicular content increased and the antioxidant level decreased in proportion to the increase in calcium chloride concentration. In the same study, when the 10% Calcium chloride solution group was compared with the 20% Calcium chloride solution group, no significant difference was found between MDA levels and antioxidant levels ($P>0.05$). In this study, no significant difference was found between pre- and post-operative TAS, TOS activities and OSI rates of castrated cats. However, it was determined that there was a decrease in TAS activity and an increase in TOS activity after castration. In this study, although there was no statistically significant difference, the decrease in TAS activity and the increase in TOS activity in the postoperative period in castrated cats were associated with the stress occurring during and after the operation.

There are studies reporting significant changes in some hemogram and leukogram values after castration. Fazio *et al.* [29] reported that they found a decrease in parameters such as RBC, HGB, MCV, and an increase in WBC and PLT levels in cats and dogs that they had ovariohysterectomy. Jana and Samanta [18] reported that they did not detect a significant change in PCV level in the cats they castrated by applying different concentrations of calcium chloride solutions intratesticularly.

Aengwanich *et al.* [2] reported that the percentage of neutrophils and lymphocytes increased significantly on the tenth postoperative day in castrated dogs. It has been reported that the neutrophil percentage of the dogs increased significantly after the recovery process was completed, and the lymphocyte percentage decreased significantly. Türkoglu [30] reported in his study that castration did not make a significant difference in the total white blood cell count of lambs. However, it has been reported that the total white blood cell count is higher than the control group. In the same study, when the castrated lambs were compared with the control group, it was reported that the percentage of lymphocytes decreased and the percentage of neutrophils increased.

Türkoğlu [30] reported that the most important effect of castration in lambs (*Ovis aries*) was the increase in the percentage of eosinophils. In this study, although the increase in WBC level of castrated cats was not statistically significant, the increase in the amount of eosinophils was statistically significant ($P<0.05$). However, although it is not statistically significant, the decrease in the percentage of neutrophils (although there is an increase in the amount of neutrophils) and the increase in the percentage of lymphocytes after the castration procedure is remarkable. Although this study is similar to some studies [30] due to the increase in the amount of eosinophils, it differs from some studies [2, 30] due to the variability in the percentage of neutrophils and lymphocytes. There are studies reporting a decrease in adrenal cortex functions in castrated animals and a corresponding decrease in the amount of glucocorticoids. In this study, the increase in the amount of eosinophils in castrated cats may be associated with the decrease in adrenal cortex activity.

CONCLUSION

Although there are many studies reporting that the castration process has positive and negative results, when the hemogram/leukogram results and the levels of oxidative stress factors were evaluated, no significant difference could be detected except for the amount of eosinophils. The significant increase in the amount of eosinophils after castration was associated with a possible decrease in adrenal cortex activity. It is known to cause a decrease in adrenal cortex functions in neutered animals. With this study, the importance of conducting more specific studies and detailing similar studies in order to evaluate the effect of castration on metabolism and the etiology of diseases has been revealed.

Financial support

This research received no grant from any funding agency/sector.

Ethical statement

This study was approved by the Firat University Animal Experiments Local Ethics Committee (dated: 22.03.2021, numbered: 28617).

Conflict of interest

The authors declared that there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Sarıerler M, Bellek CG. Surgical castration techniques and advantages and disadvantages of castration in male dogs and cats. Turkiye Klinikleri J. Vet. Sci. Obstet. Gynecol.–Special Topics. 2015; 1:52–66.

[2] Aengwanich W, Sakundech K, Chompoosan C, Tuchpramuk P, Boonsorn T. Physiological changes, pain stress, oxidative stress, and total antioxidant capacity before, during, and after castration in male dogs. J. Vet. Behav. [Internet]. 2019; 32:76–79. doi: https://doi.org/ksq9

[3] Greenfield CL, Johnson SL, Schaeffer DJ. Frequency of use of various procedures, skills, and areas of knowledge among veterinarians in private small animal exclusive or predominant practice and proficiency expected of new veterinary school graduates. J. Am. Vet. Med. Assoc. [Internet]. 2004; 224(11):1780–1787. doi: https://doi.org/csvf7h

[4] Howe LM, Slater MR, Boothe HW, Hobson HP, Holcom JL, Spann AC. Long-term outcome of gonadectomy performed at an early age or traditional age in dogs. J. Am. Vet. Med. Assoc. [Internet]. 2001; 218(2):217–221. doi: https://doi.org/dsc6h8

[5] Kustritz MVR. Early spay-neuter: clinical considerations. Vet. Clin. North Am. Small Anim. Pract. [Internet]. 2002; 17(3):124–128. doi: https://doi.org/d8pwnw

[6] Waters DJ, Shen S, Glickman LT. Life expectancy, antagonistic pleiotropy, and the testis of dogs and men. The Prostate. 2002; 43(4):272–277.

[7] Moore GE, Burkman KD, Carter MN, Peterson MR. Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993–1996). J. Am. Vet. Med. Assoc. [Internet]. 2001; 219(2):209–214. doi: https://doi.org/bhfg7w

[8] Ru G, Terracini B, Glickman LT. Host related risk factors for canine osteosarcoma. Vet. J. [Internet]. 1998; 156(1):31–39. doi: https://doi.org/bjx6gf

[9] Cooley DM, Beranek BC, Schlittler DL, Glickman NW, Glickman LT, Waters DJ. Endogenous gonadal hormone exposure and bone sarcoma risk. Cancer Epidemiol. Biomarkers Prev. 2002; 11(11):1434–1440.

[10] LeRoy BE, Lech ME. Prostatic carcinoma causing urethral obstruction and obstipation in a cat. J. Feline Med. Surg. [Internet]. 2004; 6:397–400. doi: https://doi.org/ccf83k

[11] Howe LM, Slater MR, Boothe HW, Hobson HP, Fossum TW, Spann AC, Wilkie WS. Long-term outcome of gonadectomy performed at an early age or traditional age in cats. J. Am. Vet. Med. Assoc. [Internet]. 2000; 217(11):1661–1665. doi: https://doi.org/c4gk6f

[12] Kanchuk ML, Backus RC, Calvert CC, Morris JG, Rogers QR. Neutering induces changes in food intake, body weight, plasma insulin and leptin concentrations in normal and lipoprotein lipase-deficient male cats. Nutr. J. [Internet]. 2002; 132(6):1730S–2S. doi: https://doi.org/ksrb

[13] Nguyen PG, Dumon HJ, Siliart BS, Martin LJ, Sergheraert R, Biourge VC. Effects of dietary fat and energy on body weight and composition after gonadectomy in cats. Am J. Vet. Res. [Internet]. 2004; 65(12):1708–1713. doi: https://doi.org/c6xpx9

[14] McNicholas Jr WT, Wilkens BE, Blevins WE, Snyder PW, McCabe GP, Applewhite AA, Laverty PH, Breur GJ. Spontaneous femoral capital physeal fractures in adults cats: 26 cases (1996–2001). J. Am. Vet. Med. Assoc. [Internet]. 2002; 221(12):1731–1736. doi: https://doi.org/bg6bxs

[15] Kustritz MVR. Determining the optimal age for gonadectomy of dogs and cats. J. Am. Vet. Med. Assoc. [Internet]. 2007; 231(11):1665–75. doi: https://doi.org/d6w6zg

[16] McCann TM, Simpson KE, Shaw DJ, Butt JA, Gunn- Moore DA. Feline diabetes mellitus in the UK: the prevalence within an insured cat population and a questionnaire-based putative risk factor analysis. J. Feline Med. Surg. [Internet]. 2007. 9(4):289–99. doi: https://doi.org/fmv55d

[17] Mahalingam A, Kumar N, Maiti SK, Sharma AK, Dimri U, Kataria M. Laparoscopic sterilization vs. open method sterilization in dogs: a comparison of two techniques. Turkish J. Vet. Anim. Sci. [Internet]. 2009; 33(5):427–436. doi: https://doi.org/ksrj

[18] Jana K, Samanta PK. Clinical evaluation of non-surgical sterilization of male cats with single intra-testicular injection of calcium chloride. BMC Vet. Res. [Internet]. 2011; 7(39):1–15. doi: https://doi.org/fdbmnx

[19] Piccione G, Casella S, Panzera M, Giannetto C, Fazio F. Effect of moderate treadmill exercise on some physiological parameters in untrained beagle dogs. Exp. Anim. [Internet]. 2012; 61(5):511–515. doi: https://doi.org/f4bsdp

[20] Casella S, Fazio F, Russo C, Giudice E, Piccione G. Acute phase proteins response in hunting dogs. J. Vet. Diagn. Invest. [Internet]. 2013; 25(5):577–580. doi: https://doi.org/f5gmtt

[21] Passantino A, Quartarone V, Pediliggeri MC, Rizzo M, Piccione G. Possible application of oxidative stress parameters for the evaluation of animal welfare in sheltered dogs subjected to different environmental and health conditions. J. Vet. Behav. [Internet]. 2014; 9:290–294. doi: https://doi.org/ksrk

[22] Senol O. Determination of antioxidant activity/capacity of some pharmaceutical formulation with several methods and classify pharmaceuticals by chemometric calculations. [doctoral thesis], Erzurum, Turkey: Atatürk University; 2015. 99 p.

[23] Nenadovic K, Vucinic M, Radenkovic–Damnjanovic B, Jankovic L, Teodorovic R, Voslarova E, Becskei Z. Cortisol concentration, pain and sedation scale in free roaming dogs treated with carprofen after ovariohysterectomy. Vet. World. [Internet]. 2017; 10(8):888–894. doi: https://doi.org/gnr7k9

[24] Polat E, Han MC, Kaya E, Yilmaz S, Kayapinar SD, Coskun S, Yildirim A, Can UK. The effect of hip dysplasia on some biochemical parameters, oxidative stress factors and hematocrit levels in dogs. Pol. J. Vet. Sci. [Internet]. 2021; 24(4):473–478. doi: https://doi.org/ksrm

[25] Topal A. Veterinary Anesthesia. 5th ed. Istanbul, Motif Publishing. 2005; p 43–184.

[26] Erenler AS, Karabulut AB, Sevimli R, Geckil H, Akpolat N, Unver T, Otlu O. Biochemical effect evaluation of microbial chondroitin sulphate in experimental knee osteoarthritis model. Fresenius Environ. Bull. 2022; 31:510–516.

[27] Sümbüloğlu K, Sümbüloğlu V. Biostatistic. 19th. ed.. Ankara: Alp Ofset Publishing; 2019; p 129–144.

[28] Mogheiseh A, Koohi F, Nazifi S, Tabrizi AS, Taheri P, Salavati S. Oxidative–antioxidative status and hepatic and renal factors following Melatonin administration in castrated and intact dogs. Basic Clin. Androl. [Internet]. 2019; 29(14):1–11. doi: https://doi.org/ksrn

[29] Fazio E, Medica P, Cravana C, Pupillo A, Ferlazzo A. Effects of ovariohysterectomy in dogs and cats on adrenocortical, haematological and behavioural parameters. Acta Sci. Vet. 2015; 43:1–8.

[30] Türkoglu A. The effects of castration and eııogenous testosterone hormone on the levels of serum glucose, cholesterol and creatinine in Akkaraman ram lambs. Selçuk Univ. Vet. Fak. Derg. 1985; 1:101–107.