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ORIGINAL ARTICLE

Comparative Analysis of Oxidative Stress Induced by Chemical and Surgical Castration in Male Dogs

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Oxidative Stress in Chemically vs. Surgically Castrated Male Dogs

Abstract

This study was conducted to investigate the effects of chemical and surgical castration on oxidative stress in male dogs. A total of ten healthy male dogs, aged between 1 and 3 years, were assigned to two distinct groups: Group Surg underwent surgical castration, while Group Chem received chemical castration utilizing zinc gluconate. Blood samples were collected at baseline, as well as at 1 day, 1 week, and 2 weeks following the castration procedures, in order to measure indicators of oxidative stress. The parameters assessed included total antioxidant capacity, superoxide dismutase, and malondialdehyde activities. The results indicated an increase in malondialdehyde levels immediately following both procedures, suggesting an elevation in oxidative stress. Conversely, superoxide dismutase levels exhibited a significant increase in the Surg group, indicating a robust antioxidant response. Total antioxidant capacity levels remained consistent and unchanged across both groups; however, superoxide dismutase activity was observed to be less active following chemical castration compared to surgical castration. Both surgical and chemical castration methods resulted in increased oxidative levels in male dogs, with the chemical process demonstrating a more pronounced effect by elevating malondialdehyde levels and diminishing antioxidant capacity. In summary, although total antioxidant capacity, superoxide dismutase, and malondialdehyde levels differ significantly between castrated and non-castrated dogs in both surgical and chemical methods, chemical castration imposes less oxidative stress on dogs than surgical methods. The results of this study have significant implications for post-castration care and treatment in dogs, as well as for the selection of castration methods. This may include the administration of antioxidants to mitigate the increase of free radicals.

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Introduction

Sterilization is a vital interventional method in veterinary medicine aimed at managing the dog population by rendering an animal's reproductive glands ineffective. This procedure can be performed through minimally invasive surgery or targeted chemical injections. In male dogs, castration involves the removal of the testes, which is known to significantly influence temperament, a fact supported by veterinary surgeons and animal shelter organizations (1, 2). Chemical castration is often considered a safer alternative to surgical techniques, as it carries a lower risk of severe postoperative complications, such as sperm granuloma formation, making it a more cost-effective and time-efficient option (3). However, both surgical and chemical castration can induce physiological stress, leading to hepatic oxidative stress, which is defined as an imbalance between reactive oxygen species (ROS) and endogenous antioxidants. Oxidative stress is a recognized condition that can be triggered by surgical stress, psychological stress, and tissue damage, all of which tend to elevate ROS levels (4, 5). Increased ROS levels can damage cells and are implicated in various pathological conditions, including cancer, cardiovascular diseases, and male infertility (6). To combat ROS, the body employs complex antioxidant mechanisms, utilizing enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (7, 8). Malondialdehyde (MDA) serves as a widely used marker of oxidative stress, reflecting cellular damage due to lipid peroxidation (9). Monitoring MDA, total antioxidant capacity (TAC), and SOD levels is essential for assessing oxidative stress in dogs following castration. Understanding the oxidative stress response is crucial, as chronic oxidative stress can diminish quality of life and increase the likelihood of negative outcomes, such as impaired healing and postoperative complications (10).

Scientific findings regarding oxidative stress and antioxidant status before, during, and after castration in male dogs are limited. Therefore, the present study aimed to examine some oxidative stress markers in male dogs that underwent either chemical or surgical castration. By evaluating these specific biomarkers, valuable insights can be gained into the physiological effects associated with each castration method and their respective indices of oxidative stress. Given that castration is a prevalent surgical procedure for dogs, a deeper understanding of oxidative stress can assist in designing intervention strategies that minimize surgical stress and promote recovery in dogs.

Materials and Methods

Animals and Study Design

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The dogs involved were selected from a local shelter, and all procedures related to the study were clearly explained to ensure transparency. To minimize pain and distress, all animals received appropriate veterinary care throughout the study. After castration, the dogs were kept in separate cages under identical conditions until the end of the study. Both groups of dogs were also tagged for identification before being returned to the shelter at the end of the study to avoid confusion with other unneutered dogs at the shelter. The research employed an experimental comparative design to investigate the impact of chemical and surgical castration on the antioxidant profile of the dogs, specifically measuring TAC, SOD and MDA. This study was conducted from September 4 to October 9, 2023.

A total of 10 healthy male dogs, aged 1 to 3 years and weighing 15 to 35 kg, in natural body condition, and without a history of chemical or surgical castration, were enrolled in this study. The dogs were housed for one week to acclimatize to their new environment, personnel, and food. During this period, they also received treatments for dermal and gastrointestinal parasites. This acclimatization process aimed to reduce stress and allow the blood parameters to stabilize. The dogs were divided into two groups of five: Group Surg underwent surgical castration, while Group Chem received chemical castration. To minimize inter-group variability, dogs in both groups were matched for age, weight, and breed. Before the intervention, each dog underwent a comprehensive veterinary examination, which included a complete blood count (CBC), serum biochemistry, and a physical check-up to confirm they met the inclusion criteria. Body weight and body condition score (BCS) were recorded, and blood samples were collected to establish baseline measurements of TAC, SOD, and MDA levels.

Intervention Procedures

Surgical Castration (Group Surg)

Group Surg dogs underwent surgical castration through closed orchiectomy, performed under total intravenous anesthesia (TIVA) with acepromazine maleate (1%, 0.1 mg/kg, IM, Alfasan, Woerden, The Netherlands) as premedication, followed by the induction and maintenance of general anesthesia through a combination of diazepam (0.5%, 0.2 mg/kg IV, Chemi Darou, Tehran, Iran) and

ketamine HCl (10%, 10 mg/kg IV, Bremer, Germany). The procedure utilized a closed pre-scrotal approach for testicular retrieval, ensuring aseptic conditions and keeping the scrotum outside the surgical field. Surgical dissection allowed for the retrieval of the testis without incising the tunica vaginalis, and ligatures were applied to secure the spermatic cord. Careful handling was emphasized to prevent bleeding and maintain the integrity of surrounding structures (Figure 1). Postoperative care included the administration of analgesics and antibiotics, with monitoring for complications until the dogs fully recovered from anesthesia (11).

Chemical Castration (Group Chem)

Chemical castration for Group Chem was conducted using zinc gluconate (97%; B23022, Alfa Aesar, Massachusetts, U.S.). The drug was provided with plastic calipers and instructions for measuring the width of each testicle, along with a dosing chart that correlated testicular width (10 to 27 mm) with the volume of zinc gluconate (0.2 to 1.0 mL). In this project, dogs of all ages were treated according to the product label recommendations for injection volume. Dogs with testes measuring less than 10 mm in width received 0.1 mL of zinc gluconate per testicle, while those with testes larger than 27 mm were limited to a maximum of 1.0 mL per testicle. The scrotum was gently cleaned with soap and water, avoiding scrubbing and alcohol application. Two doses were prepared for each dog using tuberculin syringes with a 5/8-inch 28-gauge needle, with the volume in each syringe calculated based on the testicular width measurement. The needle was inserted into the dorsocranial aspect of each testicle and directed caudally to ensure the tip was at the center of the testis (Figure 2), allowing for gentle injection of the solution (12). The effects of chemical castration were monitored, and follow-up tests were scheduled to evaluate the treatment's effectiveness.

Blood Sampling

Four blood samples were collected from each dog at four different time points: baseline (before the intervention), 1 day after the intervention, 1 week after the intervention, and 2 weeks after the intervention. Blood sampling was conducted through venipuncture of the cephalic vein and processed for serum separation within one hour of collection. Following centrifugation at 3,000 rpm for 10 minutes at 4 °C, the serum was separated and subsequently frozen at -80 °C until analysis.

Oxidative Stress Markers

Total Antioxidant Capacity (TAC)

TAC was assessed using a ferric-reducing antioxidant power (FRAP) assay (No. SH0148, BT Lab, Shanghai, China). In this procedure, serum samples were mixed with FRAP reagent, which consists of ferric chloride solution, 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ), and acetate buffer. The absorbance of the mixture was measured at 593 nm using a spectrophotometer. The results were expressed as millimoles of Fe^{2+} per liter of serum. The TAC concentration was determined from a standard curve and reported in nmol/mL (13, 14).

Superoxide Dismutase (SOD)

SOD catalyzes the conversion of superoxide radicals (O_2^-) into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) in a sporadic manner, making it an essential antioxidant defense in nearly all oxygen-exposed living cells. The assay for SOD is based on its enzymatic activity, which facilitates the disproportionation of superoxide anions. This reaction can be inhibited by SOD, and its activity is negatively correlated with the amount of formazan dye produced. Therefore, SOD activity can be measured through spectrophotometric analysis using the WST-1 method. The laboratory assay kit (No. SH0039, BT Lab, Shanghai, China) was employed, and the results were expressed as units per milliliter (u/mL) (14, 15).

Malondialdehyde (MDA)

MDA tests were performed using a commercial kit (No. SH0020, BT Lab, Shanghai, China). This assay employed an ELISA format with a colorimetric detection method and a quantitative measurement time of 50 minutes. The final product of oxidative damage to thiobarbituric acid reactive substances (TBARS) primarily consists of MDA, which is generated during the degradation of lipid peroxidation products. The assay quantified the elevation in TBARS levels, with the results reported in nanomoles per milliliter (nmol/mL (14, 16).

Statistical Analyses

The analysis used SPSS version 22 statistical software. A Two-Way Repeated Measures ANOVA evaluated changes in TAC, SOD, and MDA levels across three time points within and between groups, with pairwise comparisons via the Bonferroni posthoc test. Model assumptions were

confirmed using the Shapiro-Wilk test, Levene's test, and Box's test, with statistical significance set at $p < 0.05$.

Results

The lowest MDA levels in both groups were observed before castration, which was significantly different from the levels at other time points ($p < 0.05$). The highest MDA levels occurred 1 day after castration, significantly different from the levels observed at 1 and 2 weeks post-castration. Overall, the Chem group consistently exhibited higher MDA levels than the Surg group across all time points, although the difference was not statistically significant ($p > 0.05$).

Figure 1. Surgical castration in group Surg dogs through closed orchiectomy using the pre-scrotal approach for testicular retrieval. A ligature was applied to secure the spermatic cord.

In the Surg group, the lowest SOD levels were observed 2 weeks post-castration, significantly different from the highest levels recorded 1 week post-castration ($p < 0.05$). Conversely, in the Chem group, the lowest SOD levels were noted before castration, while the highest levels were observed 1 week post-castration. Notably, there was a significant difference in SOD levels between the two groups ($p < 0.05$).

The lowest TAC levels in both groups were observed one day after castration, which was significantly different from the levels at other time points ($p < 0.05$). The highest levels were recorded two weeks post-castration; however, these levels were not significantly different from those observed before castration or one week post-castration ($p > 0.05$). Both groups exhibited similar patterns of TAC changes over time, with no significant differences noted ($p > 0.05$).

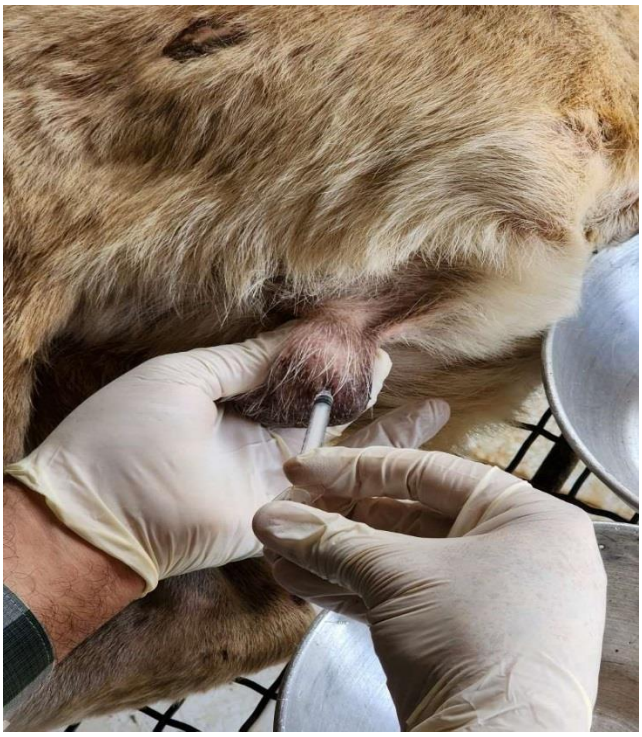

Figure 2. Chemical castration for group Chem using zinc gluconate. The needle was inserted into the dorsocranial aspect of each testicle and directed caudally to ensure the tip was at the center of the testis, allowing for gentle injection of the solution.

While MDA levels peaked shortly after the procedure, indicating oxidative stress, SOD levels suggested a more robust antioxidant response in the Surg group. Nevertheless,

Table 1. Serum MDA, SOD, and TAC levels in surgical and chemical groups

Parameter	Group	Before castration	1 day after castration	1 week after castration	2 weeks after castration
MDA (nmol/mL)	Surgical	1.62 ± 0.07^{a}	8.42 ± 0.4^{b}	6.6 ± 0.44^{c}	5.5 ± 0.38^{c}
	Chemical	1.88 ± 0.1^{a}	8.78 ± 0.19^{b}	7.04 ± 0.32^{c}	6.84 ± 0.23^{c}
SOD (U/mL)	Surgical	161 ± 5.41^{a}*	165.4 ± 7.15^{a}	221.6 ± 17.59^{b}	147.4 ± 5.09^{a}*
	Chemical	134.6 ± 7.61^{a}	167 ± 3.75^{b}	210 ± 3.54^{c}	178.4 ± 7.37^{b}
TAC (U/mL)	Surgical	58.2 ± 4.53^{a}	36.2 ± 1.96^{b}	58.4 ± 1.29^{a}	70 ± 4.17^{a}
	Chemical	44 ± 3.12^{a}	35.6 ± 2.29^{b}	56 ± 1.52^{a}	58 ± 3.7^{a}

SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity. $^{a, b}$: different letters in each row indicate significant difference ($p < 0.05$). * indicates statistically significant difference between groups ($p < 0.05$).

TAC levels did not differ significantly between the groups, indicating that TAC remains relatively stable regardless of the sterilization method. Overall, this study demonstrates that there were no significant differences in any parameters, except for SOD, between surgically castrated dogs and those that underwent chemical castration (Table 1).

Discussion

Managing free-ranging dog populations is essential due to their potential to transmit zoonotic diseases, posing significant public health risks that require effective management strategies (17). While surgical sterilization remains the gold standard for effectiveness, non-surgical methods present a viable alternative, particularly in resource-limited settings. Researchers are increasingly advocating for non-surgical sterilization methods, which are cost-effective and minimize postoperative complications. These methods, such as the use of calcium chloride or calcium gluconate, have shown promise in achieving permanent sterilization in male animals. Non-surgical methods are generally less expensive than surgical options, allowing for large-scale sterilization efforts (17, 18). These techniques enable quick treatment of numerous animals, addressing population control efficiently (19).

Both surgical and chemical castrations may have complications. Oxidative stress is one of the complications that may not be given much attention at first. ROS are an inevitable by-product of oxygen metabolism, and their cellular concentration is determined by the balance between the rate of synthesis and clearance by various antioxidant compounds and enzymes. For a long time, ROS were thought to cause exclusively toxic effects that were associated with various pathological conditions, including carcinogenesis, neurodegeneration, diabetes, and aging. However, to date, it has been shown that while prolonged exposure to high concentrations of ROS may lead to various disorders, low concentrations of ROS exert beneficial effects in regulating cellular signaling cascades (20). Reactive oxygen species attack macromolecules, including proteins, DNA, and lipids, causing damage to cellular tissue. To counteract their effects, the body produces compounds known as antioxidants. These antioxidants are either produced endogenously or obtained from exogenous sources, including enzymes such as SOD, CAT, GPx and glutathione reductase (GR), minerals such as selenium, manganese, copper, and zinc, and vitamins such as A, C, and E. Other compounds with antioxidant activity include glutathione, flavonoids, bilirubin, and uric acid. In a healthy body, prooxidants and antioxidants maintain a ratio, and a shift in this ratio towards prooxidants causes oxidative stress. This oxidative stress may be mild or severe, depending on the degree of change, and is the cause of many diseases such as cardiovascular, neurological, renal, metabolic, inflammatory, dermatological, respiratory, and hepatic diseases, as well as malignancies and aging. Oxidative stress may play a role in carcinogenesis and also influence the morbidity and mortality of patients with veterinary cancer (21).

In a study conducted by Aengwanich *et al*., the effects of time on physiological changes, pain stress, oxidative stress, and total antioxidant capacity before, during, and after surgical castration of dogs were investigated. The TAC measured before castration, after the dogs' recovery, and on day 3 was significantly lower than on day 14 of the experimental period. This study demonstrated that male dogs experienced the highest levels of pain after recovery. At that time, they were under stress, but not oxidative stress, as their antioxidant system remained highly effective. Consequently, following the recovery from castration, the dogs exhibited pain stress, as indicated by increased pain scores, elevated neutrophil percentages, higher neutrophil-to-lymphocyte ratios, increased heart rates, and elevated respiratory rates, while the percentage of lymphocytes decreased. Conversely, the dogs did not experience oxidative stress, as their antioxidant system continued to function effectively (22). However, in our study, the levels of antioxidants and ROS fluctuated over time, indicating oxidative stress in these dogs following sterilization.
Soleimanzadeh *et al*. evaluated silver-doped carbon dots (AgCDs) as a novel agent for chemical castration using a rat model and concluded that AgCDs could be considered a potent and efficient agent for chemical castration, offering a less invasive, cost-effective solution with potential applications for population control. They reported that the high-dose AgCDs group significantly reduced testosterone levels, sperm concentration, and motility, resulting in a decreased fertility index. MDA significantly increased, while SOD and TAC significantly reduced in the chemically castrated groups (23). We used zinc gluconate for this purpose and found that these parameters increased in a two-week period after the procedure.

The use of zinc gluconate for chemical castration in animals, particularly male donkeys and dogs, has been explored as an alternative to surgical methods. This approach aims to induce sterility through intra-testicular injections, with varying outcomes and implications for animal health. Studies indicate that zinc gluconate can lead to significant histopathological changes in testicular tissue, such as necrosis of spermatogenic epithelium and interstitial fibrosis, although it does not significantly reduce serum

testosterone levels in donkeys (24). In dogs, chemical castration with zinc gluconate has shown comparable outcomes to surgical methods, with fewer complications like wound dehiscence, which could be a problem when employing surgical methods (12, 25). Rafatmah *et al*. used zinc gluconate for chemical sterilization in animals, particularly dogs, through intratesticular injection. They reported it induces testicular degeneration, disrupting spermatogenesis while preserving testosterone production and general health, making it a less invasive alternative to surgical castration (26).

Despite the promising results of zinc gluconate as a chemical sterilant, concerns regarding its safety profile and the potential for adverse effects necessitate further research. The balance between effective sterilization and animal welfare remains a critical consideration in the adoption of this method. Research highlights potential risks associated with zinc gluconate, including mutagenicity at higher concentrations, suggesting that careful dosing is crucial (27). While chemical castration appears feasible, it necessitates proper follow-up care to manage potential complications such as swelling and inflammation (25).

The comparison of oxidative stress between chemical and surgical castration reveals significant differences in physiological responses. Research indicates that chemical castration, particularly through methods like $CaCl_2$ injection, results in higher cortisol levels and altered lipid profiles, suggesting increased oxidative stress and heightened stress responses, compared to surgical castration, which appears to evoke less stress and maintain a more stable oxidant/antioxidant balance. In fact, surgical castration shows a decrease in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, while chemical castration results in increased high-density lipoprotein cholesterol (HDL-C) levels (28). In rats, surgical castration resulted in increased levels of MDA and nitric oxide (NO), both markers of oxidative stress, alongside decreased testosterone levels (29). Our results confirm the previous reports and demonstrate that chemical castration using zinc gluconate could exert oxidative stress comparable to the surgical method.

The oxidative stress induced by chemical castration may have implications for long-term health, particularly in the context of conditions like prostate cancer, where oxidative stress can influence treatment resistance (30). Conversely, while chemical castration may be less invasive and cost-effective, it's potential to induce higher oxidative stress raises concerns about its long-term physiological impacts compared to the more stable outcomes associated with surgical castration (31).

The observed oxidative stress responses highlight the necessity for veterinarians to be aware of the potential oxidative status associated with different castration methods. Understanding the biochemical changes that occur can inform preoperative and postoperative care strategies, including the consideration of antioxidant supplementation to mitigate oxidative stress. Dogs undergoing surgeries such as castration may benefit from antioxidant therapies, particularly because these therapies can help reduce their susceptibility to postoperative complications associated with increased oxidative stress. By enhancing antioxidant defenses, veterinarians may improve recovery outcomes and overall health. Furthermore, the choice between chemical or surgical castration should be based on the individual dog's health status, taking into account the implications of oxidative stress.

Conclusion

In conclusion, although TAC, SOD, and MDA levels differ significantly between castrated and non-castrated dogs (before castration in both groups) in both surgical and chemical methods, chemical castration imposes less oxidative stress on dogs than surgical methods. The results of this study have significant implications for post-castration care and treatment in dogs, as well as for the selection of castration methods. This may include the administration of antioxidants to mitigate the increase of free radicals.

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Author Contributions

Dorrin Azari Motlagh: Led the experimental design, performed the chemical castration procedures, collected and analyzed data on oxidative stress, and prepared the initial manuscript draft. **Reza Asadpour**: Assisted in data collection, helped with biochemical assays for oxidative stress markers, contributed to data interpretation and manuscript revision. **Seyed Hossein Jarolmasjed**: Conducted surgical castration procedures, oversaw veterinary care of dogs, helped with methodological design, and contributed to manuscript writing. **Mohammad Hassan Khadem Ansari**: Provided expertise in biochemical analysis, helped perform statistical analyses, contributed in drafting and critical revision of the manuscript. **Siamak Kazemi Darabadi** (corresponding

author): Conceived and supervised the study, secured funding, coordinated research activities, led writing, and finalized the manuscript for publication.

Data availability

All data analyzed during this study are included in this published article.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interest

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

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