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Evaluation of the application and effects of intratesticular use of vaseline and acetylsalicylic acid 30% + vaseline for chemical castration in male rats

Evaluación de la aplicación y efectos del uso intratesticular de vaselina y mezcla de ácido acetilsalicílico + vaselina al 30 % para fines de castración química en ratas machos

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ABSTRACT

The aim of this study was to evaluate the application and effects of intratesticular use of a mixture containing vaseline and 30% acetylsalicylic acid for chemical castration in male rats. Twenty-eight male albino Wistar rats were divided into four groups: control, sham, vaseline, and vaseline + 30% salicylic acid mixture. The control group (K) received no injection, the Sham group (S) was injected with 1 mL of saline, the vaseline group (V) was injected with 1 mL of vaseline, and the vaseline + acetylsalicylic acid group (V+ASA) was injected with 1 mL of a mixture containing 30% acetylsalicylic acid and vaseline. A statistically significant difference (P=0.000) was determined between the vaseline (V) and vaseline + acetylsalicylic acid (V+ASA) groups. The Malondialdehyde (MDA) value of the vaseline group was found to be higher (3.197 ± 0.08) with a statistically significant difference (P=0.000) compared to the other groups. In terms of glutathione (GSH) value, a statistically significant difference (P<0.05) was observed in the V+ASA group compared to the V and K groups. When comparing glutathione peroxidase (GSH-Px) levels. There was a significant difference (P=0.013) between the vaseline (V) and control (K) groups. A statistically significant difference (P=0.046) was found between the vaseline (V) and vaseline + acetylsalicylic acid (V+ASA) groups in terms of catalase (CAT) activities. In terms of motility in spermatological examination, it was determined that the vaseline (V) group had the lowest rate with 11,250 ± 3.14, showing a statistically significant difference (P=0.001) compared to the other three groups. The group with the highest sperm concentration was K (82,000 ± 6.60), while the lowest concentration was observed in group V (27,600 ± 3.54). The dead/viable ratio in semen was found to be the lowest (7,200 ± 2.03) in group K and the highest (61,250 ± 13.16) in group V. The highest rate and statistical difference in a chromosome values were determined in the vaseline (V) group. Histopathologically, the morphology of the testicular tubules was impaired in the vaseline (V) and vaseline + acetylsalicylic acid (V+ASA) groups, observed as degenerated and necrotic. Degenerated tubules were observed, devoid of germinative epithelial cells and consisting only of basal membrane. In conclusion, the castration process was performed irreversibly in the vaseline (V) and vaseline + acetylsalicylic acid (V+ASA) groups.

Key words: Chemical castration; vaseline; acetylsalicylic acid; oxidative parameters; rat

RESUMEN

El objetivo de este estudio fue evaluar la aplicación y los efectos del uso intratesticular de una mezcla que contiene vaselina y ácido acetilsalicílico al 30% para la castración química en ratas macho. Veintiocho ratas Wistar albinas macho se dividieron en cuatro grupos: control, simulado, vaselina y mezcla de vaselina + ácido salicílico al 30%. El grupo de control (K) no recibió ninguna inyección, al grupo simulado (S) se le inyectó 1 mL de solución salina, al grupo de vaselina (V) se le inyectó 1 mL de vaselina, y al grupo de vaselina + ácido acetilsalicílico (V+ASA) se le inyectó 1 mL de una mezcla que contenía ácido acetilsalicílico al 30% y vaselina. Se determinó una diferencia estadística altamente significativa (P=0.000) entre los grupos de vaselina (V) y vaselina + ácido acetilsalicílico (V+ASA). El valor de malondialdehído (MDA) del grupo de vaselina fue encontrado más alto (3.197 ± 0.08) con una diferencia estadística altamente significativa (P=0.000) en comparación con los otros grupos. En términos del valor de glutatión (GSH), se observó una diferencia estadística significativa (P<0.05) en el grupo V+ASA en comparación con los grupos V y K. Al comparar los niveles de glutatión peroxidasa (GSH-Px), se encontró una diferencia altamente significativa (P=0.013) entre los grupos de vaselina (V) y control (K). Se encontró una diferencia estadística significativa (P=0.046) entre los grupos de vaselina (V) y vaselina + ácido acetilsalicílico (V+ASA) en términos de actividades de catalasa (CAT). En términos de motilidad en el examen espermatológico, se determinó que el grupo de vaselina (V) tenía la tasa más baja con 11,250 ± 3.14, mostrando una diferencia estadística altamente significativa (P=0.001) en comparación con los otros tres grupos. El grupo con la mayor concentración de esperma fue el grupo K (82,000 ± 6.60), mientras que la concentración más baja se observó en el grupo V (27,600 ± 3.54). La proporción de esperma muerto/viable en el semen se encontró más baja (7,200 ± 2.03) en el grupo K y más alta (61,250 ± 13.16) en el grupo V. La tasa más alta y la diferencia estadística en los valores de un cromosoma se determinaron en el grupo de vaselina (V). Histopatológicamente, la morfología de los túbulos testiculares se vio afectada en los grupos de vaselina (V) y vaselina + ácido acetilsalicílico (V+ASA), observada como degenerada y necrótica. Se observaron túbulos degenerados, desprovistos de células epiteliales germinativas y compuestos solo por la membrana basal. En conclusión, el proceso de castración se realizó de manera irreversible en los grupos de vaselina (V) y vaselina + ácido acetilsalicílico (V+ASA).

Palabras clave: Castración química; vaselina; ácido acetilsalicílico; parámetros oxidativos; rata

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INTRODUCTION

Street animals contribute to the emergence of undesirable situations in public and public health [1, 2]. Studies on the sterilization of pet animals continue to be relevant for animal health and welfare, as well as for community health, peace, and human dignity [3]. Castration has been considered the most effective method of controlling pet populations from ancient times to the present [3, 4, 5, 6, 7]. There are many surgical and chemical sterilization technologies. For chemical castration to be applied as an alternative to surgical sterilization, it is required that the chemical be highly successful in the castration process, have high safety, and exhibit a permanent and irreversible effect with a single application [8, 9, 10].

Chemical castration should be an economical option that minimizes the disadvantages of sterilization such as experience, equipment, cost, and compensations after surgery and surgery. Many chemical substances have been used for the purpose of castration. For example; In dogs (*Canis lupus familiaris*), solutions containing active ingredients such as methallibure, dexamethasone, metyrapone, niridazole, alpha-chlorohydrin, or danazol have been reported [11], solutions with glycerol and ethanol [12, 13, 14], zinc gluconate [15], calcium chloride [16, 17, 18], clove oil [19], $CaCl_2$ [16], tannic acid [20], lidocaine [21], in cattle (*Bos taurus*), chlorhexidine [22], in rats (*Rattus rattus*), hypertonic sodium chloride [23, 24], in cats (*Felis catus*), calcium chloride [25, 26], in minks (*Neovison vison*), zinc gluconate [27], in rabbits (*Lepus lepus*), bupivacaine hydrochloride and calcium chloride [28], in goats (*Capra aegagrus*), calcium chloride [25], in rats, 20% hypertonic sodium chloride solution [29], cadmium chloride in lambs (*Ovis aries*) [30], and in donkeys (*Equus asinus*), calcium chloride [31], have been reported.

Although we determined that vaseline is used to enlarge the muscle and penis structure in men and to enlarge the legs, hips and breasts in women [32, 33, 34], we could not find any information about its use for castration purposes. However, the complications reported in these notifications consider the potential use of vaseline for castration purposes. The fact that vaseline is very economical and easily accessible was considered an important advantage and this study was designed.

MATERIALS AND METHODS

Preparation of vaseline

Fifty g of vaseline was taken into a glass jar, The glass jar filled with vaseline was sterilized in an autoclave. Then, one mL vaseline was filled into syringes using special filling containers under antisepsis conditions.

Preparation of vaseline + 30% Acetyl salicylic acid mixture

Thirty-five g of vaseline and 15 grams of acetylsalicylic acid were taken into a glass jar and mixed. The mixture-filled jar was sterilized in an autoclave. Using special filling containers, 1 mL of the mixture was then filled into syringes.

Animal material

In the study; 28 Wistar (*Rattus norvegicus*) albino male rats, 8-9 months old and with a live weight of 330-440 g, obtained from HMKÜ Experimental Animal Center, were used. Experimental applications were carried out in accordance with the care and use conditions of laboratory animals (12 h of light-12 h of darkness and 21±1°C). Rats were provided with standard commercial feed (pellet feed) and tap water *ad libitum* during the experimental applications. Rats were divided into 4 groups of 7. The rats were kept under control for 10 days before the application and for 8 weeks afterwards. Factors such as health status, social behavior, eating and drinking of the subjects in all four groups were monitored before, during and after the procedure. After intratesticular injection, the subjects were monitored for 8 weeks, and at the end of the 8th week, samples were taken under anesthesia, euthanized with a high dose of anesthesia and laboratory examinations began immediately.

The Control Group (C) underwent routine clinical examination and was left in place without any injection. After routine clinical examination, 1 mL isotonic (0.9% NaCl solution) was injected into each testicle with a sterile syringe in the SHAM Group (S). vaseline Group (V); After routine clinical examination, 1 mL of vaseline was injected into each testicle under sedation. In the vaseline + 30% acetylsalicylic acid Group (V+ASA), 1 mL vaseline + 30% acetylsalicylic acid was injected into each testicle following the examination. Before intratesticular injection, rats were taken under Xylazine sedation with a dose of 10 mg·kg^{-1}. For three groups (excluding the K group), a prophylactic antibiotic treatment was administered for 5 days. After the procedures, individuals in all groups were macroscopically inspected for clinical and scrotal changes using the inspection and palpation method on the first, third, fifth, seventh days, and weekly repetitions.

Completion of the Experimental Study

After all controls and measurements were conducted at the end of the 8th week, the rats were anesthetized {ketamine (60 mg·kg^{-1} IM) + xylazine (10 mg·kg^{-1} IM)}, blood samples were taken, and they were castrated and sacrificed using surgical procedures.

Laboratory analyses

Oxidative stress and antioxidant markers

Malondialdehyde (MDA) levels was used for marker of lipid peroxidation, for antioxidants markers, reduced glutathione (GSH) levels and catalase (CAT) and glutathione peroxidase (GSH.Px) enzyme activity measurements were performed of the testis tissues obtained after the experimental procedure. MDA level was measured according to the method of Placer *et al*. [35], and GSH level was determined according to the method specified by Sedlak and Lindsay [36]. GSH. Px enzyme activity was performed according to the method specified by Lawrence and Burk and the results expressed as U·g^{-1} protein [37]. CAT enzyme activity was measured according to Aebi's method and results were given as g protein [38].

Spermatozoa analysis

The spermatozoa were examined using the procedure described the method by Türk *et al*. [39] ended of the 8th week. The concentration of spermatozoa in the right caudal epididymis tissue was identified using hemocytometry. Spermatozoa motility was measured using freshly isolated left caudal epididymis tissue. A phase-contrast microscope with a heating plate (37°C) was used to determine the percent sperm motility (Nikon, E-200, USA). Live-dead spermatozoa were stained with eosin-nigrosine (1.67% eosin, 10% nigrosine, and 0.1 M sodium citrate) and stained with Hancock solution to measure the percentage of morphologically abnormal sperm. The slides were then examined under a light microscope with 400× magnification. On each slide, 400 sperm were evaluated, and overall spermatozoa abnormality rates were represented as a percentage.

Histopathological examination

Testicular tissues were collected after the orchiectomy and preserved in a 10% buffered formaldehyde solution for 48 h. Tissue samples were dehydrated through alcohol series (70%, 80%, 90%, 100%), and they were then cleared in a xylol series before being blocked in paraffin. On a microtome, serial sections of 4–5 µm thickness were cut from these blocks (Leica, RM 2135, USA). Each rat's testicular tissue samples were stained with hematoxylin–eosin and the appearance of spermatogenic cells in the seminiferous tubules were studied under a light microscope (Olympus CX21, Tokyo, Japan), and the microphotographs of the results were scored (Olympus DP12, Tokyo, Japan) using Testicular Biopsy Score technique [40].

Hormone Analyses

FSH, LH and Testosterone levels were determined by analyzing in the laboratories of Baran Medical (Baran Medical LTD. STI. Ankara, Türkiye). Commercially available kits (Relassay, Türkiye)

Statistical analysis

Shapiro–Wilk normality analysis was conducted to determine normal distribution. One-way analysis of variance (ANOVA) was used to compare group means, and Tukey's test was used to determine the differences between groups. IBM SPSS version 23.0 software was used for statistical analysis, and the results as $P<0.05$ were considered to be statistically significant. All values were expressed as mean and standard error of the mean (± S.E.M.).

RESULTS AND DISCUSSION

Clinical Findings

All groups were healthy before the procedure, and no changes in eating, drinking, or social behavior were observed. Additionally, no clinical symptoms or pathological findings were detected. In the post-intratesticular injection period, individuals in all groups (except the K group) exhibited testicular edema and color changes in the scrotal skin, ranging from mild discoloration to bruising. The color change (bruising) in the swollen area became fully apparent on the 3rd d after injection. The bruising was limited to a specific area of the scrotum, did not cover the entire scrotum, and did not reach the inguinal skin. On the 7th d of the application, it was determined that the bruising had completely disappeared, and the skin had returned to its previous healthy state.

In addition to these clinical signs, only in the V and V+K groups, small masses ranging from the size of a small lentil to that of a large kidney bean were detected (FIG. 1). It was observed that the cysts did not restrict the movement of the animals and did not cause pain. Cysts were found in some animals as single masses, while others had two or three. For the larger masses, a diagnosis of vaseline cyst was confirmed by performing punctures under asepsis and antisepsis in five subjects. No need for puncture was observed for the other smaller cysts that did not bother the subjects. Upon autopsy, it was determined that all masses were vaseline cysts.

Throughout the 8-week process of the study, no signs of pain, bleeding, changes in eating and drinking habits, itching, aggression, biting the region, restlessness, lethargy, or weakness were observed in any group of animals. The well-being of the animals was deemed intact, and no signs of infection were noted.

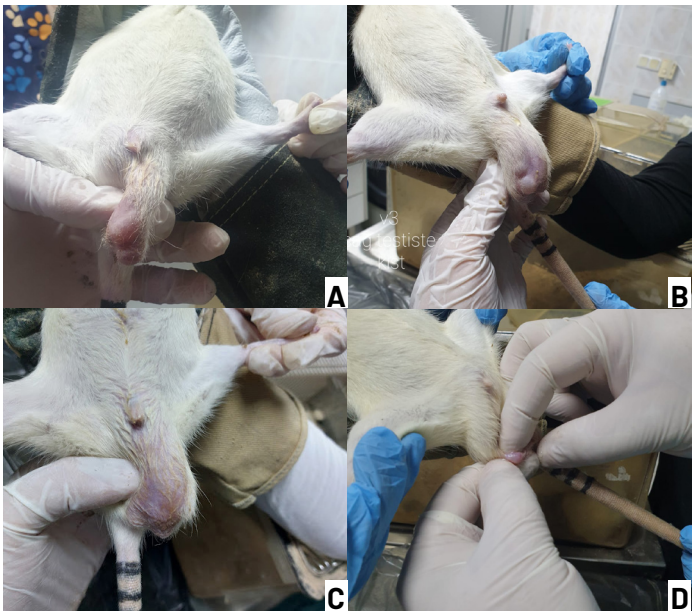

FIGURE 1. Clinical findings after injection: multicystic structures in the testicles (A and B); bruising in the testicle (C and D); Draining vaseline from cystic formation

One of the important problems of today is the constant increase in the dog population and stray dogs. This pet population, which cannot be subjected to health checks and lives in harmony with society without being vaccinated, is a very important potential danger to both human and animal health in terms of zoonotic diseases [33, 34]. To date, worldwide castration is almost the only method for animal center control [33]. Researchers are constantly striving to achieve the best methods in the castration process [20, 41]. Surgical sterilization methods require a lot of equipment, experienced personnel and financial means, and adequate post-operative care conditions, and inadequate conditions can even cost the lives of animals due to infection [8].

In the literature; solutions containing the active ingredient methallibure, dexamethasone, metopyrone, niridazole, alpha-chlorohydrin or danazol [11], glycerol and ethanol [12, 14, 41], zinc gluconate-based a solution [15], calcium chloride [16, 17, 18, 24], clove oil [19], $CaCl_2$ salt [16], Tannic acid [20], lidocaine [21], chlorhexidine in cattle [22], hypertonic sodium chloride [23], calcium chloride in cats [15, 42], zinc gluconate in bears [27], bupivacaine hydrochloride and calcium chloride in rabbits [28], calcium chloride in goats [25], 20% hypertonic sodium chloride solution in rats [29], cadmium chloride in lambs [42], calcium chloride in donkeys [31], Ligature castration has been reported [41]. Calcium chloride cannot be considered an alternative method to surgical castration. It has been reported that more research is necessary to discover other chemical sterilants with fewer complications and higher effectiveness [3, 31].

However; It has been reported that chemical castration causes aseptic inflammation in the testicular parenchyma, disrupts testicular function and achieves the purpose of castration, but although Although it is an effective method, side effects such as muscle wasting, anemia, osteoporosis and depression have been reported [43]. It has been reported in the literatüre that intratesticular injections of more than 75 IU cause granulomatous reactions [38, 39], local or systemic reactions, scrotal ulceration or dermatitis occur [8, 46, 47]; biting of the scrotum by the subject, preputial

swelling, vomiting, diarrhea, anorexia, lethargy and leukocytosis occur [8]. In the literatures, no information has been found regarding the use of V and V+ASA for chemical castration purposes. Our study demonstrated the originality and uniqueness of our approach by achieving successful results with intratesticular administration of V and V+ASA for castration purposes. The most practical and ideal agent for castration has been determined as vaseline. Because, vaseline has been evaluated both the most economical chemical agent applied up to our time, and no reaction other than local reactions (edema, discoloration, sebaceous cyst) lasting three to four days has been observed in animals. In this study, it has been determined that intratesticular injection of V and V+ASA is a method that non–invasive, most suitable, most economical, most humane, most practical, widely applicable, and minimally complicated. It is thought that this technique can be used practically by veterinarians to provide a permanent solution to the problem of stray animals.

Immunocastration is considered the best alternative technique among the currently known surgical, mechanical and technological castration technique. Gonadotropin–releasing hormone (GnRH), androgens, progestagens, anabolic steroids, and antiandrogen antagonists are used for castration purposes. However, the castration effect of these hormones is temporary and therefore they need to be repeated regularly [43]. There are numerous disadvantages to this repeated regular application: It is not cost–effective to apply to all owned and stray animals. Repeated hormonal application has negative side effects on animal health, such as obesity and immune suppression. Regular monitoring of animal owners is difficult. It has serious economic problems such as veterinary fees and medication costs. Furthermore, both the owner and the animal suffer physically and psychologically, which is seen as a significant disadvantage. In this study, none of the mentioned disadvantages occurred. In this study found that the application of V and V+ASA resulted in irreversible castration, making it one of the best alternatives to surgical castration.

Serum FSH, LH, and Testosterone levels obtained from biochemical analyses were evaluated. It was observed that the lowest levels of FSH, LH, and Testosterone occurred in Group V, and vaseline alone could achieve the desired castration effect. Although the castration effect of Group V+ASA was fully shaped as in Group V, hormone levels were found to be slightly higher compared to Group V and closer to the values of Groups K and S. Despite the determination that the lowest values in FSH and LH hormones were shaped first in Group V and then in Group V+ASA, no statistically significant difference (P>0.05) was identified between the groups.

It was determined that there was no statistically significant difference in testosterone levels between Groups S and K (P>0.05). However, in Groups V and V+ASA, a statistically significant difference was found compared to all other groups (P=0.000). The group with the lowest testosterone level was identified as Group V (1.927 ± 0.20). There was also a statistically significant difference in testosterone between Group V and V+ASA. Hormonal findings and group values are presented in TABLE I.

Intratesticular injection of glycerin and ethanol affects testosterone and spermatozoon levels and increases the pathology of spermatozoa. Intratesticular injection of zinc gluconate causes azoospermia and infertility [9, 46, 47], androgens, progestagens, GnRH, anabolic steroids, and antiandrogen antagonists have been used, but the castration effect of these hormones is temporary and requires periodic application [14, 48,], Intratesticular injection of glycerin and ethanol affects testosterone and spermatozoon levels and increases the pathology of spermatozoa [12, 14].

TABLE I
Serum FSH, LH, and Testosterone Levels (Data presented as mean ± SEM)

Group / Parameter	FSH (mIU·mL^{-1})	LH (mIU·mL^{-1})	Testosteron (ng·mL^{-1})
K	4.724 ± 0.546	30.162 ± 2.97	10.690 ± 0.67^{b}
S	4.168 ± 0.389	31.868 ± 1.22	10.858 ± 0.58^{b}
V	3.563 ± 0.230	28.726 ± 1.93	1.927 ± 0.20^{a}
V+ASA	4.571 ± 0.53	29.428 ± 2.56	7.572 ± 0.21^{c}
Significance	0.238	0.850	0.000

Different letters in the columns indicate statistically significant differences. K: control group, S: SHAM group, V: vaseline group, V+ASA: acetylsalicylic acid group

In this study, it was determined that single–dose use of V and V+ASA leads to irreversible azoospermia and infertility, without causing the reported drawbacks of hormone usage. Histologically, it was found that tubules were permanently damaged by V usage. The most effective results were obtained in terms of motility, density, acrosome integrity, dead/live ratio, testosterone levels, and sperm pathology. It has been reported that the serum concentrations of LH and FSH are higher, while testosterone hormone concentrations are lower in castrated rats following surgical procedures [49, 50]. Raman *et al.* [51] have reported that the cessation of spermatogenesis occurs as a result of necrosis in the seminiferous tubules, and due to the non–functioning of Leydig cells, testosterone levels decrease. Similarly, in the histological findings of our study, it was determined that V and V+ASA injections lysed nearly all seminiferous tubules and Leydig cells, eradicating Leydig and seminiferous tubules, and reducing testosterone levels.

In this study, FSH and LH levels with vaseline in chemical castration showed values of FSH 3.563 ± 0.230, LH 28.726 ± 1.93, which were close to and statistically insignificantly different from the control group. However, testosterone levels were determined to be at the lowest level with a value of 1.927 ± 0.20, showing a statistically significant difference. it was observed that vaseline showed a significant decrease in testosterone levels similar to surgical castration, indicating its effect similar to surgical castration and being more effective than many chemical agents. The low testosterone levels indicate that the animal may not exhibit reproductive and masculine behaviors.

Testis, malondialdehyde (MDA), reduced glutathione (GSH) levels, glutathione peroxidase (GSH–Px) and catalase (CAT) activities were evaluated. The MDA value of Group V (3.197 ± 0.08) was found to be higher than the other groups, and statistically significant differences were observed compared to the other groups (P=0.000).

There was no statistically significant difference in the MDA values among the K, S, and V+ASA groups (P>0.05). Except for the V group, the MDA values of the other three groups were very close to each other (approximately 2.5 nmol·prot^{-1}).

When comparing GSH levels, the GSH levels of the V+ASA group were found to be significantly higher than those of the V and K groups (P<0.05). The GSH levels of the V group, on the other hand, were significantly lower compared to the S and V+ASA groups (P=0.001), and a significant difference was observed between the V+ASA and S groups for the V group (P=0.001). The highest GSH value was found in the V+ASA group (1.814 ± 0.10). In the comparison of glutathione

peroxidase (GSH–Px) levels, no significant difference was found among the V, V+ASA, and S groups (P>0.05). A significant difference between the S and K groups could not be determined (P>0.05). The lowest value of GSH–Px was observed in the V group (9.211 ± 0.98). When statistically evaluating catalase (CAT) activities, a significant difference was found between the V+ASA group (P=0.046), while no statistically significant difference could be identified among the other groups. The highest CAT value was determined in the V+ASA group at 27.683 ± 2.63. Group values are displayed in TABLE II.

TABLE II
Testicular levels of malondialdehyde (MDA), reduced glutathione (GSH), and activities of glutathione peroxidase (GSH–Px) and catalase (CAT) (Data presented as mean ± SEM)

Group / Parameter	MDA (nmol·g prot^{-1})	GSH (nmol·g prot^{-1})	GSH.Px (IU·g prot^{-1})	CAT (k·g prot^{-1})
K	2.528 ± 0.14^{a}	1.398 ± 0.10^{ab}	13.629 ± 0.73^{b}	22.707 ± 1.27^{ab}
S	2.459 ± 0.11^{a}	1.645 ± 0.06^{bc}	10.423 ± 0.73^{ab}	21.666 ± 0.49^{ab}
V	3.197 ± 0.08^{b}	1.320 ± 0.06^{a}	9.211 ± 0.98^{a}	21.100 ± 1.74^{a}
V+ASA	2.544 ± 0.08^{a}	1.814 ± 0.10^{c}	12.195 ± 0.96^{ab}	27.683 ± 2.63^{b}
Significance	0.000	0.001	0.013	0.046

Different letters in the columns indicate statistically significant differences. K: control group, S: SHAM group, V: vaseline group, V+ASA: acetylsalicylic acid group

Oxidative stress occurs during surgery, it is necessary to reduce intraoperative oxidative stress for improve postoperative prognosis, however, there are insufficient reports regarding oxidative stress parameters related to castration surgery [50]. Malondialdehyde is one of the most commonly used biomarkers of oxidative stress, it has been observed that both before castration and up to the 3rd day after castration, dogs have low antioxidant capacities. This indicates that the animals are not under oxidative stress and that their antioxidant systems are effective [33]. Limited studies have been found on the effects of castration on MDA, GSH, GSH Px, and CAT values, the increase in these values compared to the control group is due to castration. The MDA value in the vaseline group is relatively higher compared to the other groups (3.197 ± 0.08), while the values among the other groups were determined to be close to each other (approximately 2.5 nmol·prot^{-1}). there were no statistically significant difference (P>0.05) between the K, S, and V+ASA groups in the study, for this reason, it was determined that the antioxidant systems in the animals were effective and there was no stress.

They reported MDA levels of 4.02 ± 0.31, GSH levels of 0.66 ± 0.08, and GSH–PX levels of 49.00 ± 5.50 in rats castrated by bilateral orchiectomy. They found that these values were higher in castrated rats compared to rats undergoing vasectomy [49, 51]. In this study, in the group castrated chemically with vaseline, they determined MDA levels to be 3.197 ± 0.08, GSH levels to be 1.32 ± 0.06, and GSH–PX levels to be 9.21 ± 0.98. In the group treated with vaseline+acetylsalicylic acid, they found MDA levels to be 2.544 ± 0.08, GSH levels to be 1.814 ± 0.10, and GSH–PX levels to be 12.195 ± 0.96. In our study, the lower antioxidant levels in both groups compared to operative castration were evaluated positively in terms of animal welfare.

Spermatological findings

Values examined in the statistical analysis of spermatological parameters (Motility, Density, Dead/Live ratio, Acrosome) were evaluated for differences between groups. In the evaluation of spermatological motility, the lowest motility value was observed in Group V (11.25 ± 3,14), while the highest value was found in Group K (80,00 ± 3,16). Statistically, while no significant difference was identified between Groups S and V+ASA (P>0.05), a statistically significant difference was observed between Group V and Groups K, S, and V+ASA (P=0.001). As a density parameter, statistical significance was found between both V and K groups and other groups (P=0.000). However, no difference was found between S and V+ASA groups (P>0.05). The group with the highest sperm density was Group K (82.00 ± 6.60), and the group with the lowest sperm concentration was Group V (27.60 ± 3.54) (TABLE III). The dead/live ratio in sperm was determined to be the lowest in Group K and the highest in Group V. Statistically, although there is a noticeable increase in dead cells within both Group K and Group S, as well as between Groups S and V+ASA, and Groups V and V+ASA, no significant statistical difference was found (P>0.05). In group V, a statistically significant difference was determined compared to groups K and S (P<0.05) (TABLE III). Although there is a mathematical difference in the V and V+ASA groups, there is no statistical difference. The lowest dead/live sperm ratio was determined in group K (7.20 ± 2.03), and the highest was determined in group V (61.25 ± 13.16). When the achromosome value in sperm is examined; While the highest value was determined in group V, the values were determined to be close to each other in the other groups. A statistically significant difference was determined between group V and other groups (P<0.019). No difference was found between S, K, V+ASA groups (P>0.05), (TABLE IV). While the highest number of achromosomes was seen in group V (17.00 ± 1.67) and the least in group K (8.40 ± 1.02), they were determined close to each other in other groups. Group V, which had the highest achromosome ratio, was considered successful. Group values are shown in TABLE III.

Intratesticular injection of chemical substances such as zinc gluconate causes infertility by causing azoospermia [8, 47, 48]. It has been reported that GnRH, androgens, progestagens, anabolic steroids, and antiandrogen antagonists are preferably used to induce azoospermia for the purpose of castration. However, since the castration effect of these hormones is temporary, the application must be repeated periodically. It has been reported that intratesticular injection of glycerol and ethanol for the purpose of castration is highly effective in reducing testosterone and spermatozoa levels. Additionally, it has been reported to significantly increase

TABLE III
Spermatological Parameters (Data are given as Mean ± SE)

Group / Parameter	Motility (%)	Density (million / right cauda epididymis)	Dead/Live (%)	Acrosome (%)
K	80.00 ± 3.16^{c}	82.00 ± 6.60^{c}	7.20 ± 2.03^{a}	8.40 ± 1.02^{a}
S	55.00 ± 2.23^{b}	9.20 ± 7.90^{b}	22.20 ± 1.35^{ab}	9.43 ± 2.202^{a}
V	11.25 ± 3.14^{a}	27.60 ± 3.54^{a}	61.25 ± 13.16^{c}	17.00 ± 1.67^{b}
V+ASA	34.29 ± 8.41^{b}	46.20 ± 3.99^{b}	45.80 ± 11.86^{bc}	9.88 ± 1.51^{a}
Significance	0.001	0.000	0.000	0.019

Different letters in the columns indicate statistically significant differences. K: control group, S: SHAM group, V: vaseline group, V+ASA: acetylsalicylic acid group

spermatozoa pathology [13, 14, 49]. In our study, we found that single-dose usage of vaseline (V) and vaseline with acetylsalicylic acid (V+ASA) leads to irreversible azoospermia and infertility. We observed no reported adverse effects similar to those associated with the use of androgens, progestagens, anabolic steroids, and antiandrogen antagonists. Particularly, vaseline was found to be highly effective in reducing testosterone levels and significantly increasing spermatozoa pathology. We also determined that intratesticular application of vaseline achieves the desired success in motility, density, acrosome integrity, and dead/live ratio of spermatozoa. Histologically, it has been determined that tubules are permanently destroyed, leading to permanent infertility. The decrease in sperm density was evaluated as a success of castration. In group V, the high rate of dead sperm was identified as a failure in reproduction

Histopathological macroscopic findings

No pathological macroscopic findings were observed in the examination of the testicles of group K and S rats. Both the V+ASA group and the V group exhibited areas in the parenchymal part of the rat testes. Some of these areas were visible through the tunica albuginea, while others appeared calcified and had a whitish color. The cut surfaces were filled with a calcified necrotic mass. It was observed that the tunica albuginea of these two groups was also significantly thicker. The macroscopic appearance of the testes of the rats belonging to the study groups is presented in FIG. 2 according to the groups.

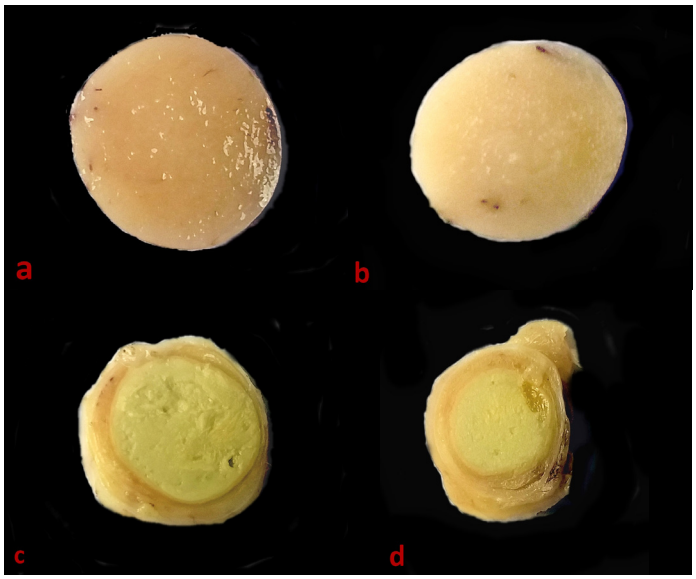

FIGURE 2. a: Normal macroscopic appearance of the testis of group K rat. b: Normal macroscopic appearance of the testis of group S rat. c: Macroscopic appearance of the parenchymal part of the testis of the V group rat, almost entirely filled with a necrotic mass, similar to the V+ASA group. d: Macroscopic view of almost the entire parenchymal part of the testis of the V+ASA group filled with a necrotic mass

Histopathological microscopic findings

In the testicular tissues of rats in group K, the basement membrane, germinative epithelium of the seminiferous tubules, Sertoli cells, and Leydig cells in the interstitial area were observed in their normal histological structures.

In the testicular tissues of rats in Group S, the basal membrane of the tubules appears normal. The histological structure is similar to the control group, with the germinal layer in the tubular wall consisting of spermatogenic cell series such as spermatogonia, spermatocytes, and spermatids, along with the supporting Sertoli cells and Leydig cells in the interstitial area and spermatogenesis continued normally.

In the microscopic examination of the testes of the rats in the V+ASA group, it was observed that the majority of the tubules were degenerated and necrotic, and their morphology was disrupted. It was observed that there were no germinative epithelial cells in the majority of degenerate and necrotic tubules, and these tubules consisted of only the basement membrane. In the degenerate tubules, it was observed that a calcified mature or necrotic mass had replaced germinal cells, and spermatognesis stopped or decreased significantly. Inflammatory cell infiltrates were detected in the interstitial area, and it was observed that the vascular structures in these areas underwent dilation, leading to congestion. In addition, it was observed that the tunica albuginea thickened several times compared to the control group due to inflammatory reaction and edema. In Group V; It was observed that the majority of the tubules in the testes of the rats were degenerated and necrotic, with deteriorated morphology. In the majority of degenerate tubules, it was observed that there were no germinal epithelial cells, or only single-tubule spermatogonia forming the stem cells, but the tubules were largely composed only of the basal membrane. It was determined that spermatognesis was largely absent. The microscopic views of the testes of rats belonging to the vaseline group are presented in FIG. 3.

Johnsen testicular biopsy score

The samples taken from the testicular tissue were stained according to the hematoxylin-eosin technique and the averages of the samples taken under the light microscope according to the Johnsen testicular biopsy score criteria are presented in FIG. 4. According to the Johnsen testicular biopsy score results, the scores of group K and group S were similar ($P \geq 0.05$), When comparing the V+ASA group with the Control group, it was statistically significantly lower in the V+ASA group ($P \leq 0.01$), It was statistically significantly lower in the V group compared to the Control group ($P \leq 0.01$), It was determined that the V+ASA group and the vaseline group were statistically similar ($P \geq 0.05$).

It has been stated that histopathological damage and atrophy occur in the testes after intratesticular administration. Additionally, the structures of seminiferous tubules are disrupted, degeneration and necrosis occur in germinal epithelial cells. Furthermore, degenerate and multinucleated giant cells are observed in the lumen of the tubules [1, 3, 12, 31, 52, 53, 54, 55, 56, 57]. The findings obtained in this study showed similarities to the findings reported by the researchers above. It was determined that the findings were more serious in these study groups. Additionally, while other studies did not provide any findings regarding the tunica albuginea, in this study, it was observed that in both groups, it was several times thicker compared to the control group.

CONCLUSIONS

As a result, in recent years, various studies have been conducted to develop a chemical sterilization method that could serve as a better alternative to surgical castration. V and V+ASA have not been previously used for chemical castration purposes. V and V+ASA were completely successful (100%) in the chemical castration procedure. However, all expected outcomes of castration were achieved in the

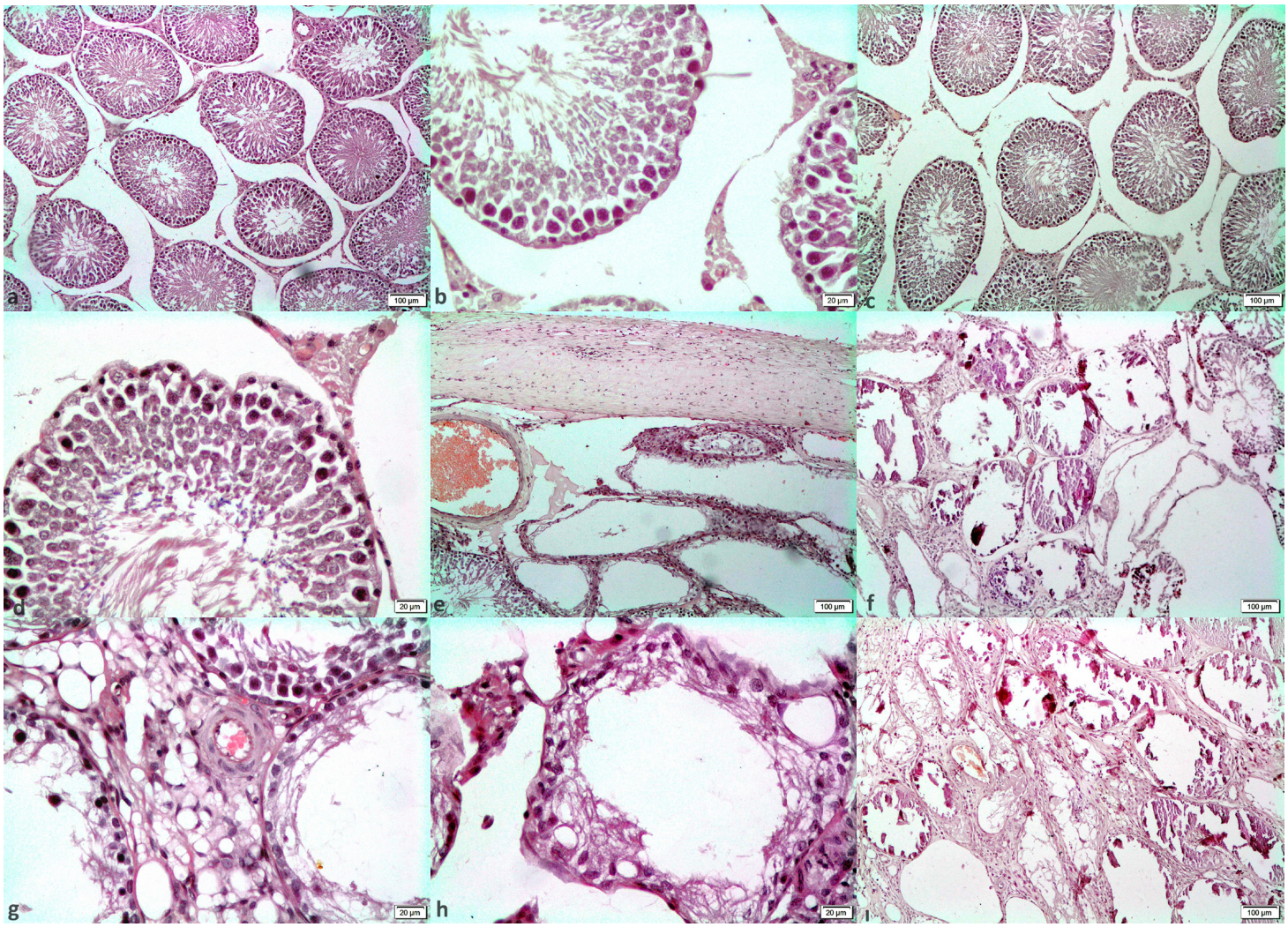

FIGURE 3. (a, b): Appearance of the normal histological structure of the testicular tissue of the Control group. H.E.; (c, d): Appearance of the normal histological structure of the testicular tissue of the Sham group. H.E.; (e): The microscopic appearance of testicular tissue belonging to group V+ASA with degenerative and necrotic changes and calcification. H.E. (f): The close microscopic view of completely distorted, degenerated, and calcified seminiferous tubules with vacuoles of varying sizes in the testicular tissue of the V+ASA group. H.E. (g): Microscopic view of the thickened tunica albuginea in the testicular tissue of group V+ASA similar to V group. H.E.; (h). Microscopic view of degenerated and calcified seminiferous tubules with completely distorted morphology in the testicular tissue belonging to the V group. H.E.; (i): The close microscopic view of pycnotic–nuclei germinal cells and Leydig cells in the testicular tissue of the V group, along with partially fewer vacuoles of varying sizes within these cells. H.E.(a, c, e, g, h: 10×; b, d, f, i: 40×)

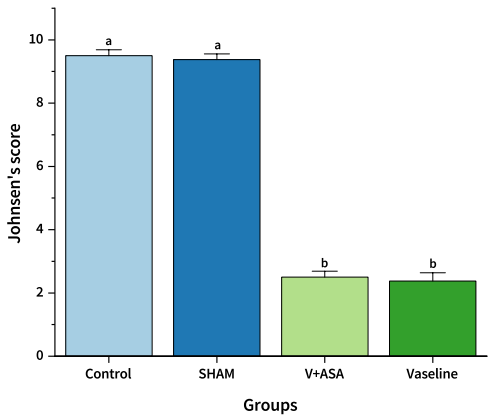

Figure 4. Evaluation of testicles according to Johnsen scores after hematoxylin–eosin staining. V+ASA: acetylsalicylic acid group

study. Therefore, it has been considered as an original, innovative, and successful study.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author (CTİ).

Conflict interests statement

The authors declare that they have no conflicting interests.

Informed consent

It is declared that this study, Permission for the study was received from HMKÜ Animal Experiments Local Ethics Committee with the decision dated 21/01/2022 and numbered 127149.

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