CHEMICAL CASTRATION WITH FORMALIN VERSUS SURGICAL CASTRATION IN DOGS: HORMONAL, SEMINAL FLUID, CELLULAR STRESS RESPONSE, AND TESTICULAR TISSUE ALTERATIONS

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ABSTRACT

Aim: Castration is almost the only way to reduce pet overpopulation. Dog overpopulation and stray dogs are global issues that harm both public health and animal welfare. As a result, the current study sought to provide alternate surgical castration approaches, if viable. Also included is a comparison between surgical and pharmacological castration.

Methods: Under the anaesthetic effect of intravenous (IV) 2% xylazine HCl (1 mg/kg) and 5% ketamine HCl (10 mg/kg), standard surgical castration and intra-testicular injections were done. The dogs were positioned dorsally recumbent. Using a 24-gauge, 2.4 cm sterile needle, a 10% formalin solution was placed within the testes (2 ml / testis).

Results: Clinical follow-up in the formalin group demonstrated edema and redness in the scrotum and prepuce following injection, according to the findings. In the formalin group, a dog developed a little scrotal ulcer. In the current study, blood testosterone concentrations in the formalin and surgery groups fell considerably at the end of the experiment compared to day 0. Cortisol levels were elevated at the start of the trial and thereafter returned to normal. Histopathological evaluation of the testes in the formalin group, showing necrosis of seminiferous tubules. In addition, oxidative stress markers rose in both the formalin and surgical groups and steadily reduced towards the conclusion of the trial. Histopathological evaluation of the testes in formalin group showing necrosis of seminiferous tubules

Conclusion: Based on the seminal and biochemical assessments in this work, intratesticular injection of 10% formalin (2 ml) seemed successful for chemical sterilization of dogs and applicable on a broad scale.

Keywords: Chemical castration, oxidative stress, formalin.

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INTRODUCTION

Castration is practically the sole method of reducing pet overpopulation. Canine overpopulation and stray dogs are a worldwide problem that endangers both public health and animal welfare (Robertson, 2008). This problem has a negative influence on environmental hygiene and zoonosis (Soto et al., 2018). Unchecked dog population growth can have a negative impact on public health and cause economic, political, and animal welfare crises (Abdulkarim et al., 2021). Homeless dogs and cats caused significant health and welfare issues, including disease transmission to livestock and humans, animal predation, bites, traffic accidents, and nuisance behavior such as barking and soiling (Macpherson and Torgerson, 2013).

Many chemicals have been used for non-surgical chemical sterilization of animal species; cadmium chloride (Murty and Sastry, 1978), calcium chloride (Abou-Khalil et al., 2020), ferric chloride and ferrous sulfate (Singh et al., 2020), glycerol (Immegart and Threlfall, 2000), zinc gluconate neutralized by arginine (Kwak and Lee, 2013), and lactic acid (Hill et al., 1985). An ideal chemical sterilizing agent would effectively inhibit spermatogenesis, androgenesis, and libido while having neither dangerous nor negative side effects (Hassan and Fromsa Merga, 2017).

The neutering effects on neoplastic and immune-mediated disorders remained significant (Hoffman et al., 2013). Chemical castration has several advantages, including a significant reduction in discomfort and stress, as well as the eradication of bleeding, hernia, infection, myiasis, and other surgical problems. It is easy, technically undemanding, and inexpensive, making it appropriate for large-scale sterilization (Ibrahim et al., 2016). Chemo-sterilant is a helpful tool, especially in areas without clinical facilities or skilled personnel (Massei and Miller, 2013, Levy et al., 2008).

As a fixative, formalin is utilized. Chemically, it interacts with the side groups of amino acids to form chemical bonds between proteins (Eltoum et al., 2001). Formaldehyde is a nucleophilic biological substance that is a formalin intermediary metabolite. It is rapidly metabolized in the mammalian body, producing formic acid, carbon dioxide, and one carbon pool, which may subsequently be incorporated into proteins or nucleic acids (Rietjens et al., 2022).

Several attempts have been made to find a promising efficacious agent, including intratesticular injections of various concentrations of formalin to induce castration in a variety of animals, including Awassi lambs (Ijaz et al., 2000), male black Bengal goats (Das and Siddiki, 2004), male Sprague Dawley rats (Osama et al., 2005), and adult bucks (Osama et al., 2005).

This study aimed to evaluate chemical castration with intratesticular injection of 10% formalin solution versus surgical castration in terms of clinical, hormonal, seminal, cellular stress response, and testicular tissue changes.

MATERIALS AND METHODS

Ethical approval
The Research Ethics Committee (REC) of the Faulty of Veterinary Medicine, Assiut University, Assiut, Egypt, has approved all the procedures in this study in accordance with the Egyptian bylaws and OIE animal welfare standards for animal care and use in research and education (NO: 06/2023/0062).

Experimental animals
This study was conducted on twelve clinically healthy adult male canines with normal descending testes (n = 12). Dogs were housed in individual standard cages and fed standard dry food (300 g/dog/day) with ad libitum access to water. They were acclimatized for one week before the study.

Experimental protocol

The dogs were divided into two experimental groups, each of six dogs (n = 6): the surgical group (Group S) and the formalin group (Group F). Dogs in the Group (S) were surgically castrated. Dogs in the group (F) received a single bilateral intratesticular injection of 10% formalin solution (formalin, Sigma Aldrich Corporation, Egypt) (2 ml/testis).

Surgical castration technique

For six hours before surgery, dogs were denied food but not drink. The dogs were given a dose of 1mg/kg xylazine HCl 2% (Xyla-Ject, ADWIA Co., SAE, Egypt). Ten min later, dogs were administered intravenous (IV) 2 % xylazine HCl (1 mg/kg) (Xyla-Ject, ADWIA Co., SAE, Egypt) and 10 mg/kg Ketamine HCl 5 % (Ketamine, Sigma-tec Pharmaceutical Industries, SAE, Egypt) in one syringe. The dogs were taken to the operating room and placed in a dorsal recumbency. The surgical area, including the scrotum and prescrotal area, was trimmed, shaved, and scrubbed multiple times with 10% povidone iodine solution (BETADINE, EL-Nile Co. for Pharmaceutical and Chemical Industries, Egypt). Except for the surgery site, the dog was aseptically dressed. According to Woodruff et al., (2015), surgical castration was conducted through the prescrotal approach.

Formalin chemical castration technique

Intra-testicular injections were performed under the anesthetic effect of intravenous (IV) 2% xylazine HCl (1 mg/kg) and 5% ketamine HCl (10 mg/kg). The dogs were held in a dorsal recumbent position. The scrotal region was shaved and disinfected several times with a 10% povidone iodine solution. Formalin solution 10% was deposited inside the testes (2 ml / testis) using a 24-gauge, 2.4 cm sterile needle. While the testis was compressed against the scrotal skin, the needle was inserted entirely along the long axis of the testis, near the head of the epididymis. The solution was deposited while withdrawing the needle from the testis. Support was made to prevent solution drainage from the injection site (Rafatmah et al., 2019). Dogs in all groups were administered a pain prescription reliever, Carprofen (Rimadyl, 50 mg/ml, Zoetis), 4 mg/kg, intravenous (IV), daily for three successive days.

Clinical inspection

For 60 days, dogs were kept under clinical supervision. Alterations in animal behavior, food or water consumption, dogs' gait, and gross changes in external genitalia were monitored and documented daily.

Blood collection

Blood samples were taken from the cephalic vein under complete aseptic condition before (0 day) and at 30 minutes, 1-, 7-, 30-, and 60-days post-castration in both groups. To separate serum, blood samples were centrifuged at 3000 rpm for 10 minutes. The serum was then collected in Eppendorf tubes and kept at 20 °C until it was analyzed. The serum was tested for testosterone, antioxidant and oxidative stress markers, liver function tests, and kidney function tests.

Testosterone assay

The serum testosterone level was tested using a rat Testosterone (T) ELISA Kit, as directed by the manufacturer (Cat. No. CSB-E05100r, Cusabio, USA). The competitive inhibition enzyme immunoassay approach is used in this assay, and the minimum detectable concentration is often less than 0.06 ng/ml.

Oxidative stress Markers
Serum cortisol assay

Throughout the investigation, serum cortisol levels were measured as a chronic discomfort indicator using an enzyme immunoassay test kit (Cat. No. CSB-E05112r, Cusabio, USA) with a minimum detectable value of 0.049ng/ml. The competitive inhibition enzyme immunoassay approach is used in this assay.

Serum malondialdehyde (MDA) assay

Malondialdehyde (MDA) was spectrophotometrically quantified using a commercially available kit (Cat. no. GR 25-29, Schiffgraben, Hannover, Germany). Malondialdehyde concentration, which is a measure of lipid peroxidation, was evaluated using a procedure developed by Janero (1990). Malondialdehyde combines with thiobarbituric acid in acidic medium at 95 °C for 30 minutes to create thiobarbituric acid. The absorbance of the resulting pink product was measured at 534 nm, and the intensity of the absorbance is related to the malondialdehyde level.

Antioxidant markers assay
Serum nitric oxide assay

The nitric oxide (NO) level was determined spectrophotometrically using a commercially available kit (Cat. no. NO 25-33, Schiffgraben, Hannover, Germany). The assay is based on the addition of Griess reagent, which converts nitrite to a deep purple azo compound. The absorbance of the azo chromophore at 540 nm precisely indicates nitrite concentration (Nims *et al.,* 1995).

Serum reduced glutathione assay

Glutathione (GSH) was spectro-photometrically quantified using a commercially available kit (Cat. no. GR 25-11, Schiffgraben, Hannover, Germany). Yellow chemical is generated when 5,5 dithiobis (2-nitrobenzoic acid) is reduced with reduced glutathione. The amount of reduced chromogen is proportional to GSH content, and its absorbance at 405 nm was determined (Noeman *et al.,* 2011).

Liver function tests
Serum total bilirubin assay

The total bilirubin level was calculated using a spectrophotometer and a commercial kit according to the manufacturer's instructions (MDSS GmbH Schiffgraben 41 30175 Hannover, Germany). When performed as directed, the minimum detection limit of this test is 1.0 mg/dL.

Serum aspartate aminotransferase assay (AST)

The aspartate aminotransferase level was determined calorimetrically using a commercial kit and the manufacturer's instructions (MDSS GmbH Schiffgraben 41 30175 Hannover, German). When performed as directed, the lowest detection limit of this assay is 7 U/L.

Serum alanine aminotransferase assay (ALT):

The alanine aminotransferase level was determined calorimetrically using a commercial kit and the manufacturer's instructions (MDSS GmbH Schiffgraben 41 30175 Hannover, Germany). When run as recommended, the minimum detection limit of this assay is 4 U/L.

Kidney function tests

Renal function was assessed using available commercial assay kits (Schiffgraben, Hannover, Germany) to measure serum creatinine (Cat. no. 234-000) and blood urea nitrogen (Cat. no. UR 21-10) according to the manufacturer's instructions. The prior parameters are spectrophotometrically assessed (Afkhami and Norooz-Asl, 2008, Toora and Rajagopal, 2002)

Semen collection and evaluation

Testicles of the surgically castrated dogs (group S) were used as a control. At the end of the trial (60 days), the testicles of the dogs in the group (F) were removed using the prescribed anesthetic regimen. Spermatozoa were collected from the epididymis (cauda) using an aspiration method with a slight modification (citrate buffer saline diluent) and a needle connected to a syringe when the organ was directly visible (Varesi *et al.,* 2013). To determine semen quality, samples were tested for sperm concentration (CON, 106/ml), motility (% motile), vital stain (% alive), and sperm morphology (% normal).

The Neubauer hemocytometer was used to determine sperm concentration. The percentage of spermatozoa with forward

progressive motility was determined in a little drop of sperm between a clean dry worm slide and a cover slip using a bright field microscope at high magnification (X 400). Normal sperms only have forward progressive motility towards the head. The eosin-nigrosin stain was used to evaluate the percentage of living spermatozoa (vital stain). The alkaline methyl violet stain is used to examine the morphology of sperm (Johnston, 1991, Shukla, 2011).

Histopathological evaluation

At the end of the study (60 days), testicles of chemically (F) induced castration were removed surgically for histopathological examination. First, the testes were subjected to macroscopical examination. Tissue samples (0.5 × 0.5) were taken from testes and fixed in 10% neutral buffered formalin before being dehydrated in successive grades of alcohol, cleaned with xylene, and embedded in paraffin. Tissue sections 4 to 5 microns thick were sectioned and stained with hematoxylin and eosin stains (H&E) for microscopical inspection (Banchroft *et al.,* 1996).

Statistical analyses:

Data for each measured parameter were tested for the normality of distributions (Shapiro–Wilk test, $p > 0.05$). Statistical significance was assessed by one way ANOVA for repeated-measures, or two-way ANOVA as appropriate. The Dunnett test and Tukey's multiple comparisons test were used for data point comparisons in each group. Data are presented as means $\pm$ SEM. Data of $p \leq 0.05$ was considered statistically significant.

RESULTS

Clinical evaluation
Chemically castrated dogs

The dogs tolerated the chemical castration with the intratesticular injection of the formalin (F). There were no recorded deaths among animals. Dogs showed discomfort and decreased appetite 3 – 6 days post-intratesticular injection, which gradually returned to normal. The dogs' gait remained unchanged.

The testes appeared enlarged and firm at the end of the intratesticular injection (Figure 1). Mild swelling in the scrotum and prepuce was observed in dogs after injection (Figure 2). The scrotal edema subsided gradually and disappeared on day 21 post-injection. Furthermore, one dog in the (F) group had scrotal ulcer (Figure 3). The ulcer was surgically treated, and healing was achieved with the 2nd intention. At the end of the study (60 days), the testes of dogs in the formalin group showed a decrease in the size (atrophy) (Figure 4).
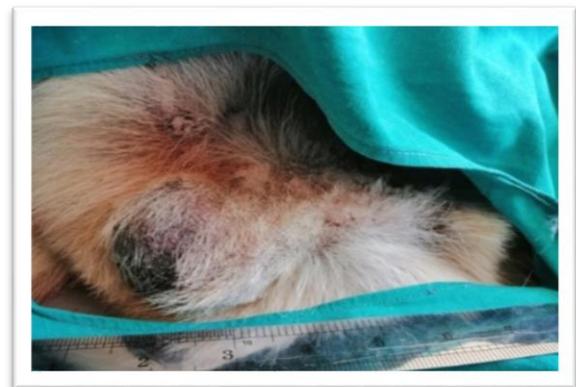

Figure (1): Testes appeared firm in consistency in the (F) group after intratesticular injection.

Figure (2): Testes revealed mild edema and hardness in consistency in the (F) group.

Figure (3): A scrotal ulcer in the formalin (F) group.

Figure (4): Testes appeared atrophied and smaller in size than normal with hardness in consistency at the end of the study (60 days).

Surgically castrated dogs:
The dogs in the surgical groups had no postsurgical problems after being castrated (Figure 5). After surgery, dogs' appetites reduced for 2 to 3 days before returning to normal. The dogs' gait remained unchanged.

Figure (5): Healing with first intention without complication in the (S) group.

Serum testosterone assay
The mean baseline value of serum testosterone concentration was (2.03 ± 0.044 ng/ml) at day 0. After surgical castration, these concentration values were characterized by a significant decrease throughout the study (30 min, and 1, 7, 30, and 60-days post-surgery) (0.63 ± 0.017, 0.02 ± 0.001, 1.06 ± 0.042, 1.20 ± 0.059, 0.02 ± 0.002 ng/ml, respectively). While in the (F) group, there was a significant decrease on day 7, returned near to normal on day 30, and then decreased again on the day 60 (0.15 ± 0.008, 0.02 ± 0.001, 1.80 ± 0.055, 2.14 ± 0.105, 1.57 ± 0.052 ng/ml, respectively). The end of the study (day 60) was characterized by a significant decrease in both (S and F), with the superiority to the (S) group compared to (F) group (0.02 ± 0.002, 1.57 ± 0.052 ng/ml, respectively) (Figure 6).
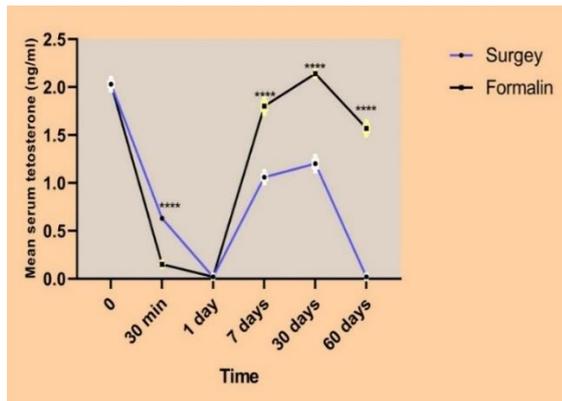
Figure (6): Values of serum testosterone for the (S) and (F) groups

Markers of the oxidative stress
Serum cortisol assay
The mean baseline value of serum cortisol concentration was (5.26 ± 0.326 ng/ml) on day 0. There was a significant increase in the cortisol level on both (S and F groups) at 30 minutes, and 1, 7, 30, 60 days post-injection (7.34 ± 0.240, 8.12 ± 0.168 & 16.56 ± 0.267, 6.35 ± 0.239 & 17.22 ± 0.124, 9.13 ± 0.181 & 17.89 ± 0.245, 6.10 ± 0.097 & 19.84 ± 0.242, 15.07 ± 0.143 ng/ml, respectively). At the end of the experiment, the (F) group was similar in their effects to the S group, where there was a significant increase in the cortisol level (15.07 ± 0.143, 19.84 ± 0.242 ng/ml, respectively) (Figure 7).
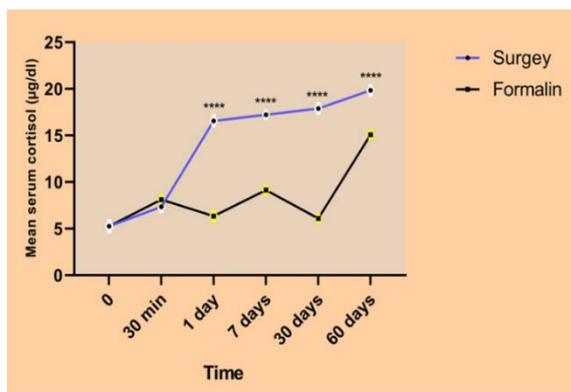

Figure (7): Values of serum cortisol for the (S) and (F) groups.

Serum malondialdehyde (MDA) assay

The mean baseline value of serum MDA concentration was (1.68 ± 0.040 nmol/ml) at day 0, which showed a significant increase in the (F) group at (30minutes, 1, 7, 30, 60

days) (4.20 ± 0.071, 8.52 ± 0.132, 6.48 ± 0.136, 5.42 ± 0.089, 2.15 ± 0.017, nmol/ml, respectively). There was a significant increase at 30 minutes, 1, and 7 days in the (S) group (5.66 ± 0.129, 7.64 ± 0.081, 2.76 ± 0.068 nmol/ml, respectively). At the end of the study (day 60), there was a significant decrease in the MDA level in the (S) group (1.17 ± 0.029 nmol/ml). In contrast, there was a significant increase in the (F) group (2.15 ± 0.017 nmol/ml) (Figure 8).

Serum nitric oxide (NO) assay
The mean baseline value of serum NO concentration was (45.00 ± 0.114 Umol/L) on day 0, which showed a significant increase in both (S and F) groups 30 min post-intratesticular injection (64.27 ± 0.170, 53.04 ± 0.037 Umol/L, respectively). Then there was a significant decrease (1, 7, 30, and 60 days) post-intratesticular injection (19.22 ± 0.171, 24.48 ± 0.194 & 19.20 ± 0.139, 24.56 ± 0.156 & 40.48 ± 0.133, 25.51 ± 0.130 & 42.41 ± 0.133, 34.58 ± 0.145 Umol/L, respectively) in both groups. At the end of the study (day 60), the effect of formalin was similar to that of the (S) group, where there was a significant decrease (34.58 ± 0.145, 42.41 ± 0.133 Umol/L, respectively) (Figure 8).

Serum reduced glutathione (GSH) assay
The mean baseline value of serum GSH concentration was (0.05 ± 0.002 mmol/L) on day 0. In the (S) group, there was a non-significant change from day 7 until the end of the study (day 60) (0.02 ± 0.003, 0.05 ± 0.019, 0.02 ± 0.002 mmol/L, respectively). While in the (F) group, recorded a non-significant change 30 min, 1, 7, and 30 days post injection (0.04 ± 0.002, 0.04 ± 0.002, 0.02 ± 0.002, 0.02 ± 0.001 mmol/L, respectively). By the end of the study (day 60), the (S) group was characterized by a non-significant difference in GSH levels (0.02 ± 0.002), while the formalin group reported a significant increase (0.09 ± 0.003mmol/L) (Figure 8).
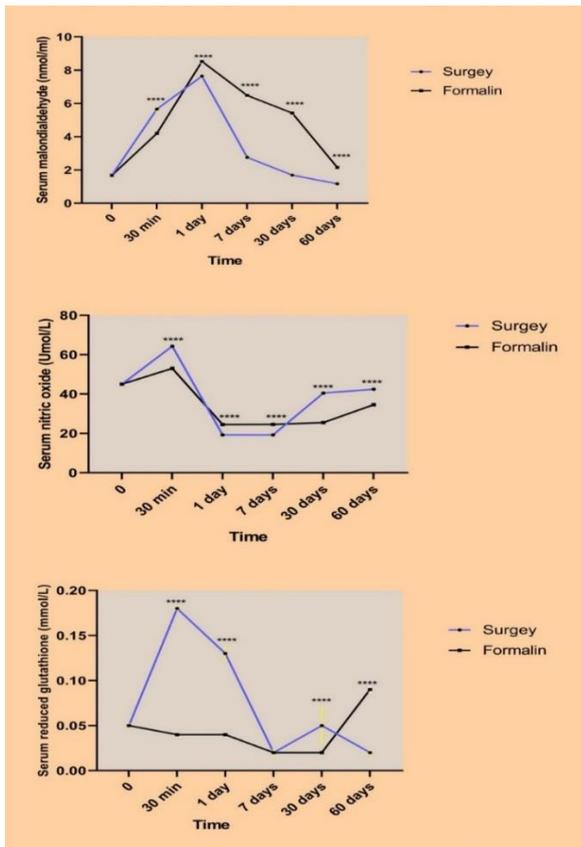
Figure (8): Values of serum MDA; NO; GSH for the (S) and (F) groups.

Liver function assays
Serum total bilirubin
The mean baseline value of serum total bilirubin (T.bil) concentration was (0.30 ± 0.007 mg/dl) on day 0. There was a significant decrease in the (S) group from (day 1) until the end of the study (day 60) (0.21 ± 0.010, 0.03 ± 0.001, 0.08 ± 0.001, 0.10 ± 0.006 mg/dl, respectively). While in the (F) group, there was a non-significant difference on 30 min and day 1 (0.30 ± 0.006, 0.30 ± 0.007mg/dl, respectively). At the end of the study (day 60), values of the (F) group were near the (S) group, characterized by a significant decrease (0.07 ± 0.001, 0.10 ± 0.006 mg/dl, respectively) (Figure 9).

Serum aspartate aminotransferase assay (AST):
The mean base line value of serum AST concentration was (25.82 ± 0.235U/L) on day 0. There was a significant increase in both (F and S) groups 30 min. and 1-day

post-intratesticular injection (30.79 ± 0.226, 43.26 ± 0.893 & 52.93 ± 0.707, 53.92 ± 0.563 U/L, respectively). Both (S and F) groups recorded a decrease from (day 7) and kept going down till the end of the study (day 60) (8.48 ± 0.169, 6.47 ± 0.194 & 25.36 ± 0.115, 10.64 ± 0.279 & 6.24 ± 0.125, 14.09 ± 0.075 U/L, respectively). At the end of the study (day 60), the (F) group was similar to that of (S) group and characterized by a significant decrease in AST level (14.09 ± 0.075, 6.24 ± 0.125 U/L, respectively) (Figure 9).

Serum alanine aminotransferase (ALT)
The mean baseline value of serum ALT concentration was (20.66 ± 0.326 U/L) on day 0 post-intratesticular injection. Both (F and S) groups showed a significant increase in 30 min and 1-day post-injection (25.97 ± 0.098, 50.94 ± 0.695 & 28.74 ± 0.225, 31.16 ± 0.511 U/L, respectively). At the end of the study (day 60), there was a non- significant difference in the (S) group compared to day 0 (19.96 ± 0.293 U/L), while the (F) group recorded a significant decrease (9.45 ± 0.140 U/L) (Figure 9).
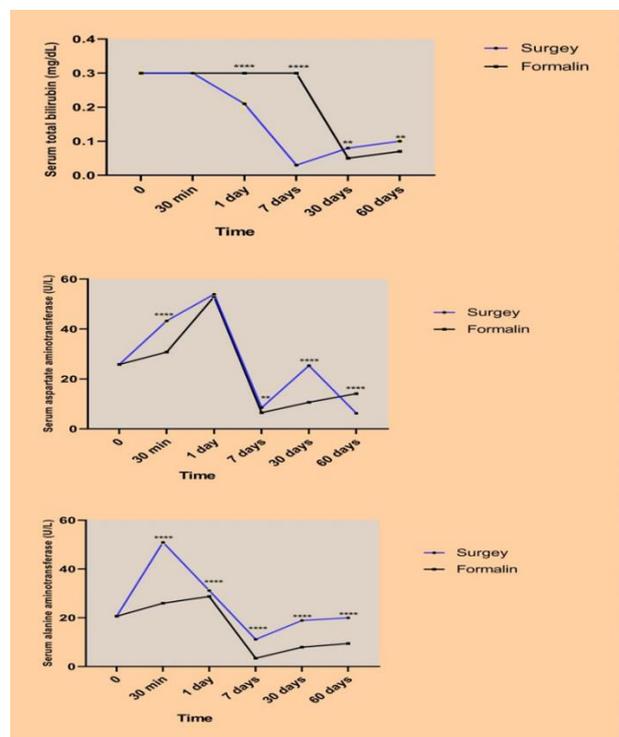

Figure (9): Values of serum TP; AST; ALT for the (S) and (F) groups.

Kidney function assays
Serum creatinine (Cr) level
The mean baseline value of serum creatinine (Cr) concentration was (1.50 ± 0.016 mg/dl) on day 0. Both (S and F) groups showed a significant decrease on 7- and 30-days post injection (0.70 ± 0.071, 1.15 ± 0.017 & 0.64 ± 0.093, 1.33 ± 0.034 mg/dl). By the end of the trial (day 60), there was a significant decrease in the (S) group (1.10 ± 0.071 mg/dl), while the (F) group showed a non-significant difference (1.10 ± 0.071 mg/dl) (Figure 10).

Serum urea nitrogen level
The mean baseline value of serum urea concentration was (28.30 ± 0.154 mg/dl) on day 0. The F group was similar in their effect to the (S) group, recording a significant increase 30 min post-intratesticular injection (33.22 ± 0.081, 45.37 ± 0.233 mg/dl, respectively). These values keep going down till the end of the study and significantly decreased (1, 7 and 30 days) in the (S and F) groups (17.36 ± 0.207, 18.41 ± 0.092 & 17.36 ± 0.161, 19.38 ± 0.111 & 19.50 ± 0.134, 20.39 ± 0.105 mg/dl, respectively). At the end of the study (day 60), the value of the (F) group was similar to the (S) group and characterized by a significant decrease (26.33 ± 0.112, 21.39 ± 0.119 mg/dl, respectively), with a greater reduction reported to the surgical group (Figure 10).
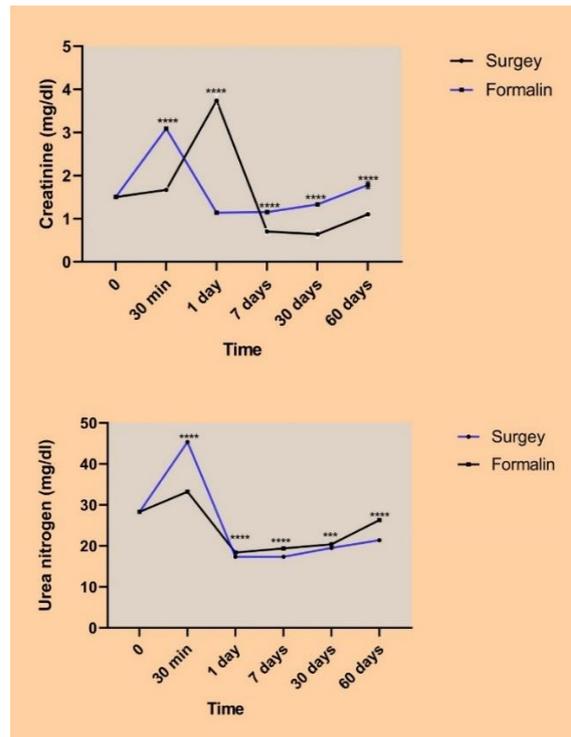

Figure (10): Values of serum urea and CR for the (S) and (F) groups.

Seminal fluid evaluation
The control parameters of the seminal fluid are reported in (Table 1) and illustrated in (Figure 11). The proportion of sperm viability and normal morphology were both high, while the percentage of motility indicated as the number of sperms demonstrating forward progression was rather low. Samples from the chemically castrated group (group F) were collected 60 days following injection, revealed no sperm in semen evaluation and depicted in (Figure 12).

Table 1: Sperm evaluation parameters (motility, morphology, viability, and concentration) of the control group

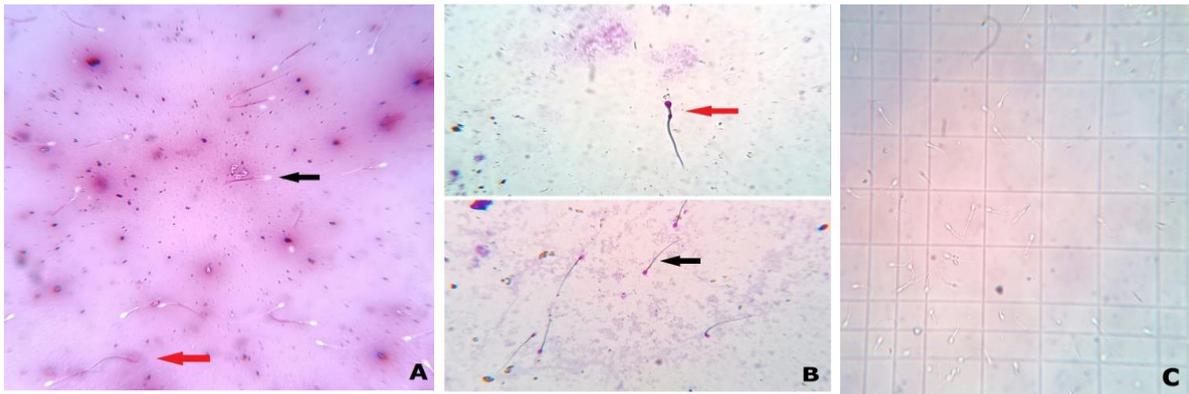| Sperm parameters	Motility %	Sperm Concentration (10^6/ml)	Morphology (normal %)	Sperm viability (alive %)
Mean Value	32.5 ± 2.5	201 ± 82	86 ± 1	94 ± 1

Figure 11: Microscopic examination of sperm in the control group, showing (A) alive sperm (black arrow) and dead sperm (red arrow) stained with Eosin nigrosin stain. (B) showing normal sperm morphology (black arrow) and abnormal sperm with distal protoplasmic droplet (red arrow) stained with alkaline methyl violet stain. (c) sperm count using Neubauer hemocytometer slide (x400).
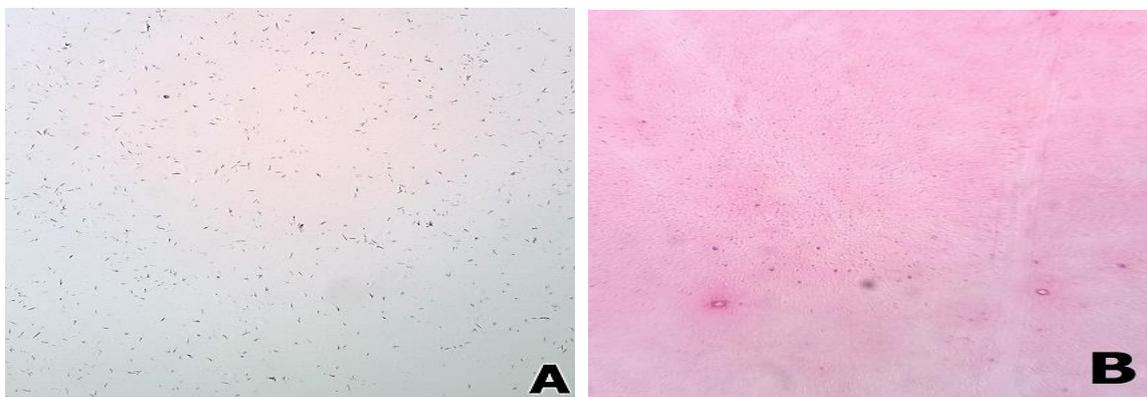

Figure 12: Microscopic examination of the (F) chemical injected group, no sperms are visible in (A) no stain and (B) Eosin nigrosin stain (x100 & x400).

Figure (13): Calcifications of the testicles in the (F) group.

Microscopical evaluation
The lesion scores of the histopathological results were summarized in (Table 2)

Histopathological examination of the testes stained by HE in the control negative group revealed normal appearance of seminiferous tubules with different stages of spermatogenesis; spermatogonia, Sertoli cells, spermatocytes, spermatid, sperms and Leydig cells (Figures 14 A, B).

Examination of formalin group showing in all cases severe pathological changes in the form of coagulative necrosis of seminiferous tubules, dystrophic calcification and hemorrhages. Interstitial fibrosis and heavy infiltration of inflammatory cells as lymphocytes (Figure15).

Table 2: Lesion score of the histopathological lesions in the studied groups.

Groups / Lesions	Control -ve	Formalin
Testicular lesions:		
Congestion of Bl.vs	-	+
Interstitial edema	-	+
Hemorrhage	-	+++
Vacuolar degeneration	-	_
Necrosis of seminiferous tubules	-	+++
Calcification	-	+++
Fibrosis	-	+++
Inflammatory cell infiltration	-	+++

Histopathological evaluation
Macroscopical evaluation

(- No lesions, + lesions present in 2-3 sections, ++ lesions present in 4-8 sections, +++ lesions present in 9-15 sections).

Grossly at day 60 in the (F) group, testes were very hard in consistency, which revealed adhesion in tunica vaginalis with the testicle with calcification (Figure 13).

DISCUSSION

When permanent sterilization of a dog is sought, surgical castration is more expensive to do on a wide scale. Therefore, there is still a need for additional effective, easy, and economic means of sterilization. Chemical sterilant for injection into the testes of dogs had been created, but they were either safe but ineffective, or vice versa. This was a motivation to conduct the current study.
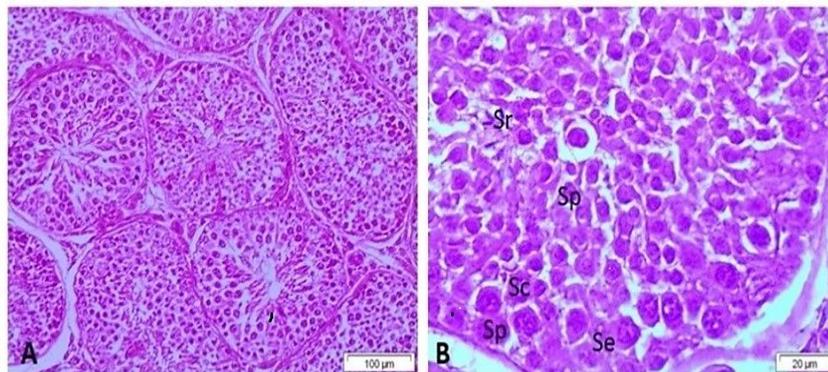

Figure (14): Representative micrograph of the testes stained by HE stain (A, B) Control negative group showing normal seminiferous tubules, spermatogonia (Sp), Sertoli (Se), spermatocytes (Sc), spermatid (Sp) and sperms (Sr).
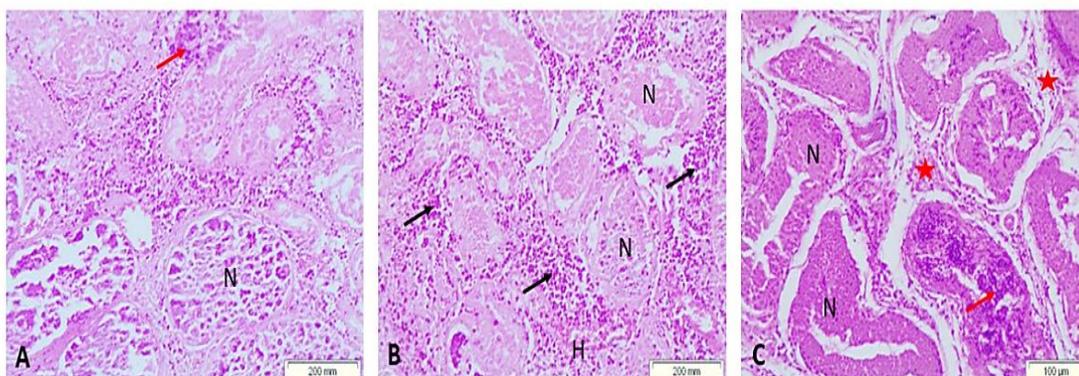

Figure (15): Representative micrograph of the testes of formalin group stained by HE stain showing necrosis of seminiferous tubules (N), calcification (red arrows), fibrosis (red star), hemorrhage (H) and mononuclear cell infiltration (black arrows).

Signs of discomfort were seen in dogs that received intratesticular injections of 10% formalin. This was in consistent with the findings of Leoci et al. (2014). Torrel et al. (1979) reported that chemical castration of rams with intra-epididymal injections of 10% formalin solution caused no discomfort. The reason for the absence of discomfort after the injection is that afferent nerve ends linked with pain feeling are situated on the scrotal skin and in the testicular capsule, rather than inside the testicular and epididymal parenchyma (Kutzler and Wood, 2006).

Because of the structure of the testes, acute testicular pain is visceral and is caused by fast pressure deforming the testicular capsule. To prevent stimulating the testicular pressure receptors during chemical castration, the injection must be administered slowly (Leoci et al., 2014). Additionally, post-injection testicular palpation revealed tenderness in all groups. The same outcomes were observed by (Majeed, 2011, Leoci et al., 2014, Canpolat et al., 2006, Al-Asadi and Al-Kadi, 2012, Abu-Ahmed and Howaida, 2015). This might be related to fluid buildup inside the testicular parenchyma (Abu-Ahmed and Howaida, 2015).

Clinical follow-up in the formalin group revealed edema and redness in the scrotum and prepuce after injection. This is consistent with Immegart and Threlfall (2000). In our investigation, formalin was applied locally to the seminiferous tubules, since it is known to promote protein denaturation in situ, as opposed to systemic toxins, which must be absorbed and transferred to distant organs to have their effects. Testicular swelling is a normal reaction to injections that cause acute inflammation (acute orchitis), which is characterized by congestion and edema, increasing forces that tend to transport fluid from the intra-vascular compartment into the interstitial tissue (Al-Asadi and Al-Kadi, 2012).

A little scrotal ulcer was detected in a dog in the formalin group. A similar finding was observed by Torrel et al. (1979), (Majeed, 2011, Al-Asadi and Al-Kadi, 2012). In another research, Immegart and Threlfall (2000) recorded scrotal ulceration following intratesticular injection of glycerol in dogs. Scrotal ulcers developed as a result of the chemical substance's irritating impact (Pandey et al., 2000). At the end of the study, testes of all dogs in the formalin group were smaller than normal. This is compatible with (Majeed, 2011, Ijaz et al., 2000, Al-Asadi and Al-Kadi, 2012). Formalin destroyed the seminiferous tubules, which are the functional units of the testis (Ijaz et al., 2000).

Desirable methods of contraception for stray male dogs need a substantial drop in testosterone levels (Leoci et al., 2014). In the current investigation, serum testosterone concentrations in the formalin group decreased significantly towards the conclusion of the trial compared to day 0. This is supported by the findings of (Al-Asadi and Al-Kadi, 2012). It appears that intra-testicular injection reduces the capacity of Leydig cells to react to LH and produce testosterone (Canpolat et al., 2006, Al-Asadi and Al-Kadi, 2012).

Stressful situations, such as castration, produce an imbalance between oxidants and antioxidants at the cellular and individual levels, resulting in oxidative stress (Aengwanich et al., 2019). The more the oxidative stress, the greater the cellular damage during surgery, which may result in poor post-operative results (Mogheiseh et al., 2019). Surgical techniques cause significant stress in animals (Nenadović et al., 2017). Cortisol has long been recognized as a stress signal (Cohen et al., 1990). In dogs, any sort of stress resulted

in an increase in cortisol release (Guyton and Hall, 1986)

At the end of the experiment, the formalin group had effects similar to the surgical group, which caused a significant increase in cortisol levels. Similarly, Mogheiseh *et al.* (2019) discovered that the elevation in cortisol lasted around 1-2 weeks following gonadectomy. Also, Abu-Ahmed and Howaida (2015) conducted a study to assess the efficacy of a single bilateral intra-testicular injection of calcium chloride or clove oil to induce chemical sterilization in dogs. They discovered that cortisol levels were elevated during the first week following calcium chloride injection. Mogheiseh *et al.* (2019) conducted research to explore gonadectomy stress, steroid hormones, and serotonin in male dogs treated with melatonin prior to gonadectomy and discovered that cortisol concentration rose considerably in gonadectomized pups compared to control dogs. While Abou-Khalil *et al.* (2020) conducted a study in donkeys to compare the effects of surgical castration versus chemical castration using calcium chloride dissolved in absolute ethanol, they discovered that the calcium chloride group had significantly higher cortisol levels at the end of the experiment than the surgical group.

The disruption of the balance between free radical emission and antioxidant enzyme reserve is commonly referred to as oxidative stress (Lykkesfeldt and Svendsen, 2007). Free radical-induced oxidative stress causes oxidation of polyunsaturated fatty acids in the erythrocyte cell membrane, leading to lipid peroxidation (Gate *et al.,* 1999). A high malondialdehyde level is a sign of lipid peroxidation, which is caused by oxidative damage. MDA, a breakdown product extensively used as a lipid hydroperoxides test, is an effective predictor of oxidative damage (Argüelles *et al.,* 2004). Nitric oxide (NO) is an indication of oxidative stress and plays an important function in the host protection and homeostasis of biological molecules such as superoxide anion and oxyhemoglobin to create nitrites and nitrates when generated at a low level for a limited period (Çiftci *et al.,* 2021).

Malondialdehyde levels were found to be higher in both the formalin and surgical groups, although they were significantly lower at the end of the trial in the surgical group. Furthermore, nitric oxide levels were increased only at the start of the study, and then decreased or returned to normal levels in both study groups. There were no significant changes in serum, reduced glutathione levels, although there was a considerable rise in both study groups at the end of the trial. Our findings contradict the findings of Aengwanich *et al.* (2019), who studied the physiological changes, pain stress, oxidative stress, and total antioxidant capacity in male dogs before, during, and after castration, and found no significant difference in MDA levels but a significant decrease in total antioxidant capacity.

Numerous studies have been conducted to examine the effects of surgical time, complication, postsurgical pain, and systemic stress parameters on oxidant-antioxidant status in the bitch after open and laparoscopic ovariectomy and ovariohysterectomy. The more oxidative stress, the greater cellular damage during surgery, which may result in poor post-operative results (Lee and Kim, 2014). As a result, any reduction in oxidative stress might be crucial (Kücükakin *et al.,* 2009). Furthermore, our findings are consistent with those of, who discovered that castration significantly increased oxidative stress by decreasing antioxidant enzymes and increasing MDA concentration. Interestingly, at the end of the current

investigation, the oxidative and antioxidant indices in the surgical group were higher than in the formalin group.

According to one study, oxidative stress and inflammation in the liver caused by aging were more pronounced in castrated female rats than in intact female rats. Castration might have both direct and indirect effects on the liver (Kireev et al., 2008). The current study found a substantial decrease in bilirubin levels at the start of the trial, but a rise in AST and ALT serum levels. By the end of the study, bilirubin, AST, and ALT serum levels had returned to normal in both the surgical and formalin groups. Our findings are comparable with those of Mogheiseh et al. (2019), who discovered that castrated dogs had considerably higher levels of hepatic liver enzymes.

Serum creatinine is an important indicator of kidney health. A blood urea nitrogen test determines the quantity of urea nitrogen in the blood. As a byproduct of protein digestion, the liver creates urea in the urea cycle (Lai et al., 2021). The current study discovered a large drop in creatinine levels at the start of the trial for both the formalin and surgical groups, but a significant rise in the formalin group by the end of the study. Furthermore, both the formalin and surgical groups' blood urea nitrogen concentrations increased at the start of the study, and then decreased until the end of the study. Our findings are similar to Mogheiseh et al. (2019) research, which found an increase in creatinine and urea levels in surgically castrated dogs. Furthermore, formaldehyde exposure causes renal dysfunction, inflammation, and redox imbalance in rats (Ramos et al., 2017).

In the current study, the microscopic evaluation of all dogs revealed no sperms in the samples collected from the tail of epididymis after 60 days of injection. Similar results were mentioned by Habeeb (2015) in local rams after 20 days of intra-testicular injection of 95% ethanol solution. On the other hand, Canpolat et al. (2006) observed that semen characteristics of only 3 bulls were very poor when using absolute ethanol.

Comparable findings were reported by Al-Asadi and Al-Kadi (2012) who injected 3% formalin intra-testicular in bucks, where the sperm concentration reached zero on day 21 post injection. Also, in Iraqi local bucks, Nahi et al. (2019) found that formalin injection caused suppression of the sperm production process. Moreover, Ijaz et al. (2000) reported that after injection of 10% formalin in Awassi lambs, no spermatozoa were observed in all spacemen collected from the epididymis.

Examination of formalin group showing in all cases severe pathological changes in the form of coagulative necrosis of seminiferous tubules, dystrophic calcification and hemorrhages. Interstitial fibrosis and heavy infiltration of inflammatory cells as lymphocytes. Incompatible with Das and Siddiki (2004), examined the effects of 10% formalin on testicular tissue of six healthy pre-pubertal male black Bengal goats (21 days old), the histological changes were not uniform. A distinct wrinkling of tunica albuginea was observed, and peripheral seminiferous tubules were more affected. Marked fibrosis in intertubular spaces was also observed. Also, Ijaz et al. (2000) studied the effect of intra-testicular injection of neutral buffered formalin on seminiferous tubules in Awassi lamb, which showed an increase in vascularity and seminiferous filled with connective tissue. Neto et al. (2014) reported that after intratesticular injection of 20% NaCl hypertonic solution, induced coagulative necrosis of Leydig

cells and seminiferous tubules and extensive testicular fibrosis.

CONCLUSION

Based on the seminal and biochemical evaluations in this study, intratesticular injection of 10% formalin (2 ml) appeared effective for chemical sterilization of dogs and capable for application on large scales.

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الإخصاء الكيميائي باستخدام الفورمالين مقابل الإخصاء الجراحي في الكلاب : التغيرات في الهرمونات والسائل المنوي ، واستجابة الإجهاد الخلوي وتغيرات أنسجة الخصية

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الإخصاء هو الطريقة الوحيدة تقريبًا لتقليل الزيادة المستمرة في أعداد الحيوانات الأليفة وبخاصة الزيادة في أعداد الكلاب الضالة والتي تعتبر من القضايا العالمية التي تضر بالصحة العامة ورفاهية الحيوان. نتيجة لذلك ، سعت الدراسة الحالية إلى توفير طرق كيميائية بديلة للإخصاء الجراحي ومقارنة نتائجهما معا.

أثناء الدراسة تم تقسيم الحيوانات الي مجموعتين، الحيوانات الموجودة بالمجموعة الأولي تم اخصائها جراحيا، في حين أن حيوانات المجموعة الثانية تم اخصائها كيميائيا عن طريق استخدام الفورمالين تركيز ١٠% وقد تم الاخصاء في كلا المجموعتين تحت تأثير التخدير.

وفقًا للنتائج أظهرت المتابعة السريرية لمجموعة الفورمالين وجود وذمة واحمرار في كيس الصفن والقلفة بعد الحقن. ايضا في مجموعة الفورمالين، أصيب كلب بقرحة صغيرة في كيس الصفن. في الدراسة الحالية ، انخفضت تركيزات هرمون التستوستيرون في الدم في مجموعات الفورمالين والجراحة بشكل كبير في نهاية التجربة مقارنة باليوم صفر. ارتفعت مستويات الكورتيزول في بداية التجربة ثم عادت إلى وضعها الطبيعي بعد ذلك.

بالإضافة إلى ذلك ، ارتفعت علامات الإجهاد التأكسدي في كل من مجموعات الفورمالين والجراحة وانخفضت بشكل مطرد حتى نهاية التجربة. التقييم النسيجي المرضي للخصيتين في مجموعة الفورمالين أظهر نخر في الأنابيب المنوية.

الخلاصة: في هذا العمل بناءً على العديد من التقييمات المختلفة، بدا أن استخدام الفورمالين بنسبة ١٠٪ ناجح في التعقيم الكيميائي للكلاب وقابل للتطبيق على نطاق واسع.