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STANDARD SURGICAL AND CALCIUM CHLORIDE (CHEMICAL) NEUTERING TECHNIQUES IN MALE DOGS: *COMPARATIVE STUDY*

By

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ABSTRACT

The present study is to determine the effectiveness of each neutering approach through clinical, laboratory, and histopathological evaluation. The results revealed that a single bilateral intra-testicular injection of calcium chloride dehydrates 20% in absolute alcohol solution is effective, economical, and easy to perform; it causes permanent sterilization and is a simple alternative method to surgical castration in control of overpopulation.

INTRODUCTION

Neutering is usually performed to prevent undesirable breeding or prevent unwanted behaviors or future medical problems **(Fossum, 2013)** and reducing the total number of unwanted puppies **(McKenzie, 2010)**. Many techniques for sterilizing dogs surgically have been described; each technique offers advantages and disadvantages to both the animal and surgeon **(Abd El- Wahed *et al.,* 2014).** Neutering has been classified according to the used technique into standard surgical/knife technique **(Fossum, 2013)**, pinhole castration "in situ spermatic cord ligation" **(Baba *et al.,* 2012)** and Non-knife technique (Chemical Neutering) that applied through intratesticular injection of a chemical substance as Calcium Chloride ($CaCl_2$) 20% **(Jana and Samanta, 2007 and 2011 and Abu Ahmed, 2015).** Regardless of the technique selected, strict adherence to sound surgical technique and asepsis is mandatory for good surgical outcome with minimal complications **(Fossum, 2013 and Abd El-Wahed *et al.,* 2014)**.Non-surgical methods for sterilization of male pets were documented by world society for protection of animals (WSPA) **(Tasker, 2008)**. The present study aimed to compare between standard surgical and chemical sterilization by intratesticular injection of Calcium Chloride techniques of male dogs through economical, clinical, laboratory and histopathological assessments.

MATERIAL AND METHODS

The present study was conducted under the regulations of the ethical committee of Faculty of Veterinary medicine, Cairo University (EAURC) code for experimental animal use.

Study Design:

Animals Grouping:

The present study was conducted on 18mongrel dogs; mixed breed, of age 1-5 years old and weighing 10-25 kg. They were housed, operated and observed at the clinic of surgery department, faculty of Veterinary Medicine, Cairo University. All dogs were vaccinated and dewormed. Experimental animals were allocated randomly into three groups (n=6).

Group I:

Control (Sham) group. *Group II:* Surgical-knife (prescrotal) sterilization group; and

Group III*:*

Chemical sterilization (Calcium Chloride) group.

Anaesthetic Regimen:

The animals of groups II were generally anaesthetized according to **(Dugd *et al.*, 2010 and Sahwan,2014)** using premedication by Atropine Sulphate 0.02–0.04 mg/kg,s.c (Atropine®100 mg/ml, ADWIA, Egypt) and Xylazin Hcl 1mg/kg.b.wt, i.m (Xyla-Ject®20mg/ml; ADWIA, Egypt). Induction and maintenance was performed by Ketamine HCl5.5 mg/kg, i.m (Ketamine®50 mg/ml, Sigma Co., Egypt). The animals of group III were only tranquilized using Atropine® and Xyla-Ject® with the same previous doses.

Sterilization Techniques:

Group one*:*

The animals were divided equally (n=3) as control (Sham group) for each technique. They were subjected to the same procedures of each sterilization method (preparation, anaesthesia). One subgroup (n=3) has subjected to all preparation steps without performing orchiectomy as sham group for surgical ervention, the second group (n=3) as sham group for chemical sterilization they were subjected to intratesticular injection of normal saline.

Group two:

The animals of this group were subjected to the sterilization via standard surgical-knife castration. The complete aseptic precautions were followed, after that, the pre-scrotal castration technique was performed according to **(Booth, 2003, Tobias, 2010 and Fossum, 2013)**.Each animal received a course of Ceftriaxone® (Ceftriaxone HCl, Alkahira Co., Egypt) 25 mg/kg 24hr for 3-5 days P.O. The surgical wound was daily inspected and dressed using Betadine solution® and antibiotic spray.

Group III:

The dogs of this group were sterilized through non-surgical/chemical sterilization method according to **(Leoci *et al.,* 2014**). Intratesticular injection of a single dose of 20% Calcium Chloride dihydrate (CaCl2) tincture solution. Calcium Chloride (CaCl2) 20% Solution were *used.* Under complete aseptic conditions, Calcium Chloride powder (2g) (Sigma Aldrich Corporation) was dissolved in a tube containing 10 ml absolute ethyl alcohol (Analal® WNR International Ltd. BH151TD England). The tube was put in a stirrer for complete dissolving of the powder and obtaining a clear solution. Technique of *Intratesticular Injection:* The animals were tranquilized and the scrotal region was aseptically prepared. A 22G needle was inserted percutaneously into the caudal pole of the testis then it was gently pushed towards the other pole; directed from the caudoventral aspect of each testis approximately 1 cm from the epidydimal tail and towards the dorsocranial aspect of that testis, depositing the injection homogenously as far as possible through the tissue. The solution was deposited in the center of the testis at a dose of 1 ml/ 10 kg.b.wt.

***Post-Operative evaluations*:**

All the experimental animals were subjected postoperatively (P.O) to the following: *Financial and Managemental Care Assessments:* All usable materials used in each sterilization technique were valued and calculated in Egyptian pounds. The effort of labour after each technique was also considered and evaluated. *Clinical Evaluation:* All animals were subjected the day after operation to daily examination for general health condition (temperature, heart rate, respiratory rate, mucous membrane and food intake). The scrotal region was daily inspected for signs of inflammation (swelling, and pain), postoperative complications as wound infection, necrosis or skin excoriation.The pain signs were evaluated as restlessness, teeth grinding, appetite condition and wound licking.

Laboratory Evaluation:

Daily for one week postoperative, a blood sample (5ml) was collected from all control and experimental animals before performing sterilization. and then once a week to the end of the study period (4weeks).The collected sample was divided in two vacutainer tubes; EDTA containing tube for complete blood picture (CBC) and plain tube for serum extraction for testosterone and cortisol hormones assessments. *Histomorphological Evaluation:* The testes

harvested from the animals of GII (surgical castration) were obtained directly postoperatively and used as a control tissue samples. While at the end of each observation period, the animals of other groups GI and GIII (Control and chemical) were subjected to surgical castration under complete general anaesthesia. The testes with epididymis and spermatic cord were collected, observed grossly and testes weresagittally sectioned for macroscopic changes, then fixed in Bruin's Fixative solution for 48 hours**(Jana and Samanta,2011),** then preserved in 10% neutral buffered formalin for at least 48 hours and then embedded in paraffin wax. A section of 5 µm thick was cut from the middle portion of each testis, stained with hematoxylin-eosinand examined under light microscopyat100X,200Xpowers **(Culling,1983)**.

Statistical analysis. The statistical analysis of the obtained data carried out using BMI SPSS program. The data was expressed as Means± SD (SPSS, ver 23, 2015).

RESULTS

Economically the standard surgical castration technique nearly the triple cost of chemical castration (Table 1).the time and effort of labor after surgical castration (30-45 minutes) was greater than chemical castration technique (15-20 minutes).

Table (1): The economic and financial value of the materials required for each sterilization technique in dogs.

Usable Materials			Bilateral orchiectomy		$CaCl_2$	
Name	**Unit**	**Price* L.E**	**Amount**	**Total price**	**Amount**	**Total price**
Atropine	**1ml**	**1.65**	**2ml**	**3.3**	**2ml**	**3.3**
Xylazine	**1ml**	**2**	**1-2ml**	**2-4**	**1-2ml**	**2-4**
Ketamine	**1ml**	**10**	**2-3ml**	**20-30**	**-**	**-**
Syringe	**1Piece**	**1**	**7**	**7**	**5**	**5**
Scalple blade	**1Piece**	**4.5**	**1**	**4.5**	**-**	**-**
Surgical Needles	**1Piece**	**1.5**	**2**	**3**	**-**	**-**
Cotton	**1Piece**	**3**	**1**	**3**	**1**	**3**
Gauze	**1Piece**	**3.5**	**1**	**3.5**	**-**	**-**
Vicryl	**1Packet**	**18**	**1**	**18**	**-**	**-**
$CaCl_2$	**1ml**	**2**	**-**	**-**	**2-6**	**4-12**
Antiseptic	**1 bottle**	**16.5**	**1**	**16.5**	**1**	**16.5**
Antibiotic local	**1 bottle**	**18.5**	**1**	**18.5**	**-**	**-**
Antibiotic systemic	**1 vial**	**11**	**4**	**55**	**-**	**-**
Total Price			**137.8-145.5**		**34.8-44.7**	

***The price listed according to the customer price approximately.**

Clinically: The intraoperative complications were not observed, except two animals of GII showed little intraoperative bleeding after spermatic cord ligation and cutting. These animals were managed by haemostasis via re-ligature of cord. While in the GIII there was, no complications recorded intraoperatively. The animals of G II showed decreased activity, lay down in sternal recumbence in their boxes for the first 2 days P.O., and back gradually to full activity at 7th day P.O. while the animals of G III showed no alterations in their activity and they were alert. In the surgical group, the body temperature was slightly increased (0.5-1°C) for the first 3 days P.O, and then it was back to normal spontaneously. While two dogs showed persistence increase for 5 days P.O, they developed wound infection signs and managed by wound dressing and continuing antibiotic course. The scrotal region examination revealed that; surgical inflammatory signs as slight redness, hotness, moderate scrotal swelling and pain in GII. In GIII, the animals showed slight testicular swelling, slight hotness, firmness and pain. The observed signs were subsided spontaneously within 7 days. One dog showed skin inflammation and necrotic spots (changing in color) at 4th day post injection. The skin developed open wounds, the animals managed by surgical-knife castration with wound dressing. Also in GIII length and width of the testis increased in its dimensions about 0.4 ± 0.2 cm at day 1 and returned to normal size at the day 7and then gradually decreased in its dimensions than control ones at the end of observation period .*Laboratory:* Significant variation in the blood parameters was not found (Tables 2, 3) except in dogs developing testicular infection that had leukocytosis indicating an inflammatory reaction. In GIII the cortisol level was nearly the same level as in control group all the time of the experiment(4.94c ±0.23 and 4.02c±0.43), while in GII high and significantly different values as compared to the control group all the time of the experiment (7.27b± 0.46 , 12.80a ± 0.74).

Table (2): Effect of Method of castration on WBC in dogs.

Day after castration	Control		Open surgical castration		Chemical		F value	P value
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*		
Day 0	**7.53**	**0.54**	**7.40**	**0.40**	**7.367**	**0.37**	**0.07**	**1.00**
Day 1	**7.71**	**0.53**	**7.77**	**0.35**	**7.90**	**0.30**	**0.24**	**0.93**
Day 7	**7.11**	**0.64**	**7.63**	**0.49**	**8.04**	**0.42**	**0.56**	**0.73**
Day 14	**7.23**	**0.47**	**7.77**	**0.44**	**8.07**	**0.60**	**0.99**	**0.46**
Day 21	**7.44**	**0.81**	**7.20**	**0.85**	**7.50**	**0.92**	**0.32**	**0.81**
Day 28	**8.08**	**1.07**	**6.97**	**0.78**	**7.57**	**0.85**	**0.42**	**0.75**

Values (Mean ±SEM, n=5/group) within the same raw with different superscripts were significantly at p<0.05

F value = is a statistic of ratio 2 different measure of variance for the data.

P value = used in hypothesis testing to help you support or reject the null hypothesis.

Table (3): Effect of Method of castration on RBC in dogs: Means (n=5/group) within the same

Day after castration	Control		Open surgical castration		Chemical		F value	P value
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*		
Day 0	**6.99**	**0.18**	**8.43**	**0.54**	**7.40**	**0.60**	**1.39**	**0.30ns**
Day 1	**6.95**	**0.27**	**8.60**	**0.53**	**6.90**	**0.90**	**1.28**	**0.33ns**
Day 7	**6.98**	**0.36**	**8.71**	**0.44**	**7.30**	**0.56**	**1.69**	**0.21ns**
Day 14	**6.49**	**0.16**	**8.69**	**0.60**	**7.73**	**0.68**	**2.47**	**0.11ns**
Day 21	**6.60**	**0.31**	**8.59**	**0.49**	**7.33**	**0.64**	**1.95**	**0.20ns**
Day 28	**7.43**	**0.25**	**8.73**	**0.52**	**7.47**	**0.72**	**0.91**	**0.48ns**
Day 28	**7.40**	**0.82**	**5.97**	**0.58**	**6.77**	**0.69**	**1.03**	**0.43ns**

Raw with different letters were significantly at p<0.05.

GIII the testosterone level from the day 7 to the end of observation period on the day 28 reached the same values of testosterone in GII (0.49 ± 0.12, 0.16 ±0.06 and 0.73± 0.20) all values in castrated animals were significantly different from control ones.

Morphohistological Evaluation:

Macroscopically; in case of control group GI, the testis appeared normal in size, texture and normal tunics with cut section there is no observable changes in parenchyma or mediastinum Fig. (1).In case of group III, the testis appeared smaller than other groups, thickened capsule with subscapular calcification due to deposition of calcium under testicular parenchyma and this calcium deposition reached to spermatic cord Fig. (2). microscopically; In GI and GII, testicular microscopical examination revealed that, normal testicular tubules and spermatogenic epithelium with slight vaculation was carried out in normal saline injected animals. The interstitial connective tissue was within normal distribution with no or little infiltration of polymorph inflammatory cells Fig (3). In GIII, microscopically we find that, histological examination of testicular tissue showed massive tubular necrosis and desquamation of germ epithelium within the lumen of atrophied tubules.There was increase in fibrous connective tissue with calcification in form of basophilic structurless necrotic debris Fig.(4). Interstitial infiltration with polymorph inflammatory cells was evident Fig (.5).

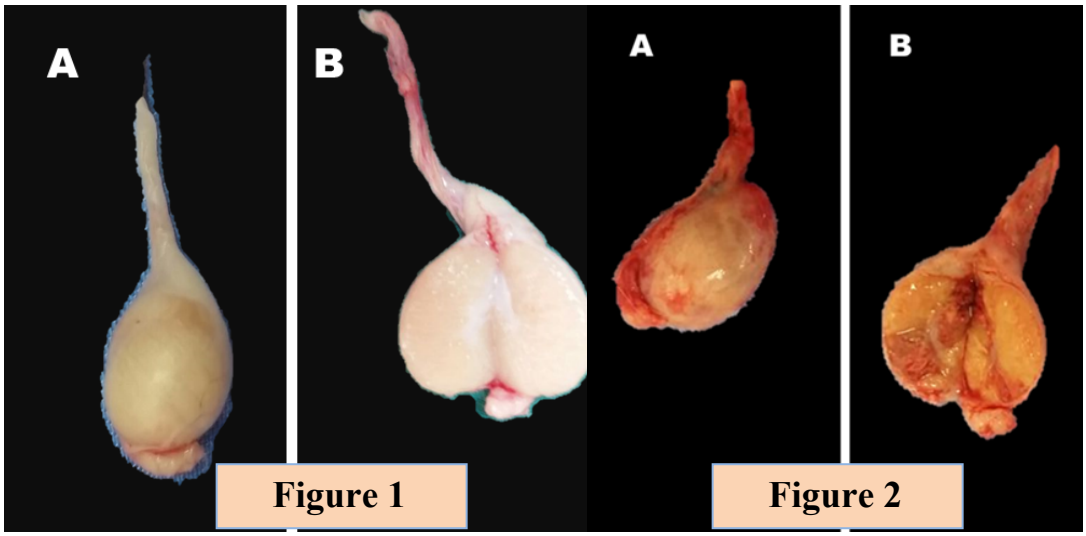

Fig (1): Morphological photograph of testes from group II before **(A)** and after **(B)** sectioning showing normal testicular size with normal parenchyma and mediastinum appearance and architecture.

Fig (2): Morphological photograph of testes from group III before **(A)** and after **(B)** sectioning showing decreased testicular size with necrotic foci in parenchyma and mediastinum.

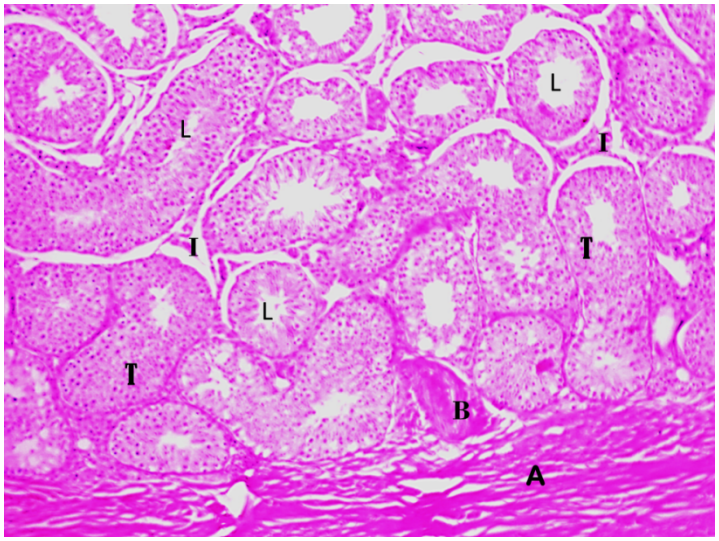

Fig (3): Microscopical photograph of testicular tissue of a dog from GI (Control) at the end of the observation period showing normal tunic albuginea **(A)** and normal testicular tubular epithelium **(T)** and lumina **(L)**. There normal interstitial connective tissue **(I)** amount without any cellular infiltration and dilated blood vessel **(B)**. **(H&E, x100).**

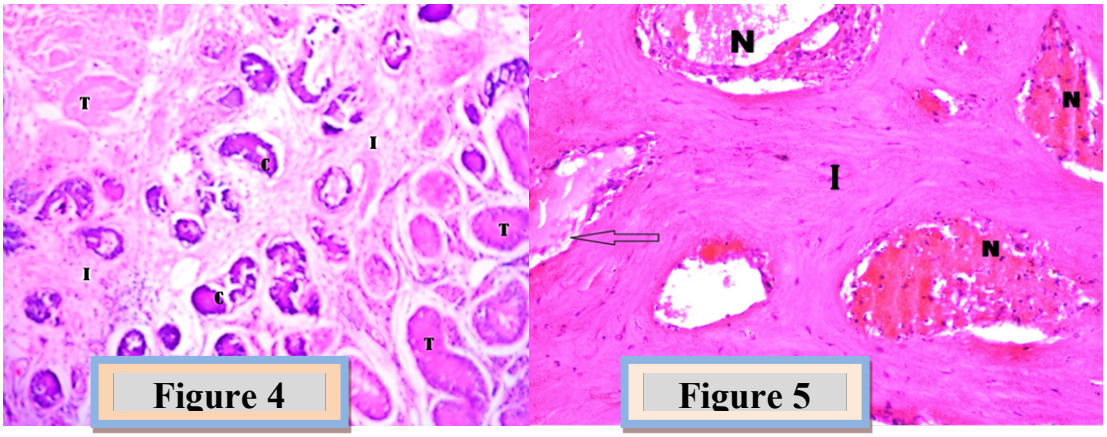

Fig. (4): Microscopical photograph of testicular tissue of a dog from GIII (Chemical) at the end of the observation period demonstrating marked atrophy of the tubular germ cells appeared as necrotic debris **(T)**, interstitial fibrosis (I) and calcification **(c)** of the seminiferous tubules. **(H&E x100).**

Fig. (5): Microscopical photograph of testicular tissue of a dog GIII (Chemical) at the end of the observation period showing severe necrosis of the spermatogenic cells at different degrees (N) with increase in the interstitial connective tissue (I) with some leucocytic infiltration and detached S.E in lamina of seminiferous tubules **(arrow) (H&E x200).**

DISCUSSION

The results of the present study revealed that, the time consumed in the standard surgical technique take (25-30 minutes) this results came in agree with that recorded by **(Okwee-Acai *et al.*, 2012 and Abd El-Wahed *et al.*, 2014)**.The chemical castration came in accordance with **(Jana and Samanta, 2007)** who recorded the technique was the least time consuming. The current study divulged that, the standard surgical sterilization technique required materials priced (137.8-145.5 Egyptian pounds). The outcomes of this study revealed that, the chemical castration had low expenses (34.8- 44.7 Egyptian pounds) rather than the radical ablation intervention. Moreover, the chemical induction did not need sutures, post-operative care including antibiotic, local or systemic, dressing and tranquilizer for suture removal concerned the effort of labour was higher in standard surgical technique, this agreed with **(Leoci, *et al.*, 2011).** As regards to anaesthesia, the applied regime was agreed with that reported by **(Dugdale, 2010)** who attributed that, this short anaesthetic period had low costs, no excitement during induction or recovery. In the surgical group (GII), the clinical observation displayed that, slightly increase in temperature, moderate decreased appetite, pronounced gait disorders, subtle elevation of heart and respiratory rate and faint increase of mucous membrane refilling time in two dogs. (Fossum, 2013) reported the same observation**,** this clinical investigation only persists for 2 days postoperatively. The animals restored its normal parameters after 3 days, the changes mentioned before were imputed to post-surgical reaction. Post-surgical complications as wound dehiscence was noticed in one orchidectomized dog and ascribed to the wound licking by himself. The observed drawbacks in the chemical group (GIII) were represented by faint discoloration of the skin of scrotum in one dog followed by formation of necrotic spot after CaCl2 20% injection 4 days later. **(Jana and Samanta, 2011)** mentioned the same complication. They put-down this findings to leakage of solution from injection hole. This problem was fixed by use one needle to draw calcium chloride out of the tube and replace the needle before injecting the testicle **(Leoci, *et al*;2014)**.They reported that every injection should be made with a fresh needle and gently pinch testicle at base of needle and hold for 5- 10 seconds to ensure safe removal of the needle while avoiding release of any CaCl2 20% into the scrotum. Moreover, remove the needle very slowly (15 to 30 seconds) to ensure that calcium

chloride remains at the center of the testicle **(Jana and Samanta, 2011)**. .Regarding the testicular swelling in GIII; it is believed that, it is due to edema followed by necrosis of the testicular tissue leading to atrophy of testicular gland parenchyma. The CaCl2-treated, as well as alcohol-treated, animals showed signs of mild increase in testicular size following injection due to the excessive fluid pressure and inflammatory reaction (necrosis) **(Jana and Samanta,2007, 2011 and 2014)**. They declared that, the minor discomforts during injection of CaCl2 dehydrate 20% and swelling noticed in a few dogs was caused by the needle and intra-testicular pressure. Afferent nerve endings associated with pain sensation are located only on the scrotal skin and in the capsule of the testis rather than within the testicular parenchyma. Therefore, these nerve endings may have been stimulated as intra-testicular pressure increased during and immediately following injection. Assay of cortisol concentration has been used as an indicator of stress and pain in dogs **(Hellyer, P.; Rodan, I.; Brunt., J.; Downing, R.; Hagedorn JE and Robertson, S.A.2007; Stafford *et al.,* 2002, Okwee-Acai *et al.,* 2012 and Azab, 2012)**. The obtained data concerning the impact of method of castration on blood cortisol level in dogs showed that; plasma cortisol assay in surgical method elicited great cortisol values as compared to the control group all the time of the experiment. In GII, plasma cortisol levels sharply decreased after two weeks and returned to normal values at the third week P.O. These results have the same opinion with **(Okwee-Acai *et al.,* 2012 and Ponvijay, 2007)**. In chemically castrated group, the cortisol values were higher than in control group in the 1st week of observation. Then became as the same values of the control group all the time. Such finding matched with that mentioned by **Jana and Samanta (2007)**; who described that cortisol concentrations in the animals treated with calcium chloride were similar to the controls as this method of sterilization does not appear to be associated with any general stress response. From Day 14 and afterwards, all castrated groups had a significant lower in blood testosterone levels than controls. This outcome was reached by **(Jana and Samanta, 2007 and 2011; Leoci, *et al,* 2014).** Using absolute Alcohol as a diluent and sterilant for calcium chloride dehydrate 20% solution, in this study, energizes the degenerative effect on the testicular tissues. **Leoci, *et al.* (2014)** said that, Alcohol alone is a chemical that causes testicular sclerosis. A study of intratesticular injection of absolute alcohol ethanol solutions of CaCl2 have demonstrated the definite advantages of more consistent efficacy,

less pain, and less peripheral inflammation. In chemical castration, group (GIII) macroscopic evaluation revealed thickened capsule with sub-capsular calcification due to deposition of calcium in testicular parenchyma that reached to spermatic cord. There was loss of conventional oval shape that became elongated with firm nodular stony texture. In cut section; testes showed macroscopic defined necrotic foci, these results were alike that noticed by (**Jana and Samanta, 2007 and 2011; Leoci, *et al,* 2014).**Histopathological examination was established as the most reliable diagnostic approach. In case of chemical castration, there are massive necrosis surrounded by fibrous connective tissue and calcification as deposition of calcium salts in form of basophilic structurless necrotic debris. Severe tubular degeneration as desquamation and detaching of epithelial cells, marked atrophy of the tubular germ cells eosnophilic and neutrophelic infiltration and there was no sign of regeneration in germ cells and interstitial Leydig cells ,the findings coincided with that mentioned by **(Jana and Samanta, 2007 and 2011; Leoci, *et al,* 2014)**. The results of this study concluded that, the chemical castration was the cheapest method for neutering dogs regarding the expenses, consumed time, less pain no need for post-operative care and need less experienced operator.

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