

Research article

Castration of Iraqi local bucks by unilateral spermatic cord torsion compared with castration by Burdizzo

Ahmed Kadhim Munahi

Thaier Alwan Abid

Department of surgery and obstetrics, College of Veterinary Medicine, / University of Al-Qadisiyah, Iraq

Corresponding Author Email: ahmed.munahi@qu.edu.iq

Co-Author Email: thaier.abid@qu.edu.iq

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Abstract

The study aimed to investigate the efficacy of using unilateral spermatic cord torsion as one of the easy castration techniques in Iraqi local black goats, also to study the effect of unilateral spermatic cord torsion on the contralateral testis. Fifteen local male black goats (adult bucks 13 - 15 months weighing 31.9 ± 3.25 Kg) were divided into three equal groups. The first group was left without treatment as control. The second group was submitted to a unilateral (right) spermatic cord torsion. The third group was submitted to bilateral castration by Burdizzo. Weighing of animals and semen were collected from bucks before castration, and after two months of castration, for seminal analysis. In addition, blood samples before castration, after two months of castration and two weeks after orchietomy were taken for determination of the serum testosterone hormone level. After two months of castration, both testes were orchied surgically in all animals for studying the weight, dimensions and histopathological changes of testes. For that, it can be used as a technique for castration since it was easy and with fewer complications. As well as the unilateral spermatic cord, torsion causes a damage to the contralateral testis.

Keywords: Castration, Buck, Spermatic cord torsion.

Introduction

Goats are a species of animals characterized by many unique biological features such as high fertility, ability to produce twins, triplet and even quadrant pregnancies, and resistance to different types of diseases. The gynecological problems are very few and were regarded as a useful model to study the urogenital abnormalities. (1). Castration means a process, which stops the function of the testes leading to sterilization. (2). The indications of castration are different according to reasons of castration such as to stop the production of male hormones and sperms, prevent mating after age of puberty, produce animal to be easier to handle with less aggressiveness, avoid unwanted pregnancies and mating of

young females before they are of adequate size and age for pregnancy and parturition and reduce gouty smell in males. (3). Castration also is an important management practice to maintain control of the breeding program which includes the removal or destruction of the testes, epididymis and portion of each spermatic cords. The common techniques, which are used for castration, are Burdizzo, elastration, ligation of spermatic cord and chemical castration. (4). Torsion of the spermatic cord causes strangulation of gonadal blood supply with subsequent testicular necrosis and atrophy. It is generally accepted that unilateral testicular torsion causes contralateral testicular deterioration and results in diminished

fertility in experimental animals. (5). Spermatic cord torsion is regarded as an emergency case with few data related with ipsilateral and contralateral changes in the testes especially of small ruminants, so this technique was used as a new manual one for castration. The objectives of the present study are to investigate the efficacy of using spermatic cord torsion as one of the easiest castration techniques, to study the effect of unilateral spermatic cord torsion on the contralateral testis of local black goats.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 417

The present study achieved in the animal teaching station of the college of veterinary medicine in the university of Al-Qadisiyah during the period extended from November 2009 to March 2010. The study was conducted on 15 local Iraqi male goats (adult bucks) (13-15months) with initial body weight (31.9 ± 3.25 Kg). All animals were healthy and free of obvious physical and clinical abnormalities. Bucks were managed under uniform housing conditions. They were allowed to graze on natural pasture during the daytime and supplemented with concentrate feed daily on average (2.5%) of their body weight (6). Water was offered freely. All bucks were dewormed with Ivermectin at dose (0.3 mg/kg). (7), and vaccinated against enterotoxaemia. All fifteen bucks were divided randomly into three equal groups. The animal's in-group one were left as a control group. Bucks in-group two were submitted to unilateral spermatic cord torsion and called (U. torsion group). With Burdizzo castrator and where called (Burdizzo group) castration the group three was done. The control group was left entire (non-castrated) to the end of experiment, after two months from castration in other groups, where complete bilateral orchiectomy was done on all the fifteen

animals of the study for histopathological examination. The animals in U. torsion group were restrained in lateral recumbency under help of Xylazine at dose (0.1-0.3 mg/kg) intramuscularly; the neck of the scrotum was prepared for aseptic surgery (Fig. 1) and anesthetized locally by ring block using lidocaine HCl 2% (8). Vertical skin incision (1.5–2 cm long) was made over the spermatic cord involving the skin and subcutaneous tissue till the tunica vaginalis which remains intact. A curved artery forceps was inserted under the right spermatic cord to expose it outside the skin incision, then twisting the cord (720°) along its longitudinal axis using tissue forceps. The torsion was maintained in position by fixing the cord to the subcutaneous tissue with one stitch silk suture (Fig. 2) (9). The skin incision was closed routinely and the post-operative care included the injection of benzyl penicillin (20 000 IU/kg BW and dihydrostreptomycin (20 mg/kg BW) per day for five days. Pain analgesic dimalgine (5ml) was administered in the first 24 hrs. after surgery. The castrated animals were kept separated from non-castrated animals until complete healing. Skin incisions were examined daily and removal of sutures was done at (14) days after surgery. (10). Bloodless technique using standard Burdizzo instrument. The procedure was applied after pushing down the testicle in the scrotum and positioning the Burdizzo at the neck of the testes over the cord followed by crushing the cord and leaving the instrument in place for approximately two minutes. Each spermatic cord can be crushed twice (Fig. 3) without crossing the midline (testicular media raphe) to minimize the risk of impairing the scrotal vascular supply. Same procedure was repeated on the other side of the spermatic cord (below or above the first one), care must be taken to be sure that only the cord was crushed and that no other organs were damaged. (11). One month before starting the experiment, bucks were trained for semen collection once a week by artificial vagina. The temperature of the

artificial vagina was between 40-43°C during semen collection. By using an induced estrus doe (via injection of estradiol in a dose of 0.1 mg/kg IM), all adult males could mount and ejaculate regularly when presented with any estrus female. The semen was collected using the artificial vagina from all adult bucks once before castration and once two months after castration. Semen assessment was done within approximately 20 minutes after collection. The evaluation of semen included macroscopic examination: Quantitative evaluation: the volume of semen and qualitative evaluation: recording color, consistency, odor, pus or blood. Microscopic examination: Physical criteria were evaluated for the collected semen kept in water bath already warmed to 37°C.

Sperm Motility:

A- Mass motility:

This procedure was done by placing the drop of fresh non-diluted semen on a warm slide 37°C and examined under a light microscope with a heater stage at 10X magnification (12).

B-Individual motility:

The examination of the semen under 40X magnification for identification of the movement and direction of the single sperm which a mark for the viability of sperm. The individual motility could be measured by putting a droplet of fresh semen on a warm stage of the microscope with a drop of sodium citrate 2.9% and placing a coverslip. The motility was scored by taking the percentage of the proceeding sperms only (13). Sperm concentration was estimated by using the formula postulated by (14). Viability of sperm was determined by (15). Testosterone Measurement (ng/ml): Blood samples (5 ml) were collected from all animals (control and castrated groups) before treatment to estimate the normal values of hormone and after two months of castration to determine the effect of castration on hormone level and two weeks later after bilateral orchietomy for serum testosterone assays. The blood samples were allowed to

clot in a refrigerator. After centrifugation (2000 rpm for 15 min), the serum was separated and stored at (-18 to -20 C°) until analyses were taken. Serum testosterone levels were determined by using Radio-immuno assay method with active testosterone RIA DSL-4000 Kit (16). All presented data were mean \pm SE. SPSS program were used to determine the difference between the mean of control and the treatment values of body weight, testes measurements, semen assessments and serum testosterone concentrations, as well as (LSD) test was used to compare the significant variances among means ($P < 0.01$). (17).



Figure (1) Preparation the site for aseptic surgery in buck.



Figure (2): Fixation of rotating cord.

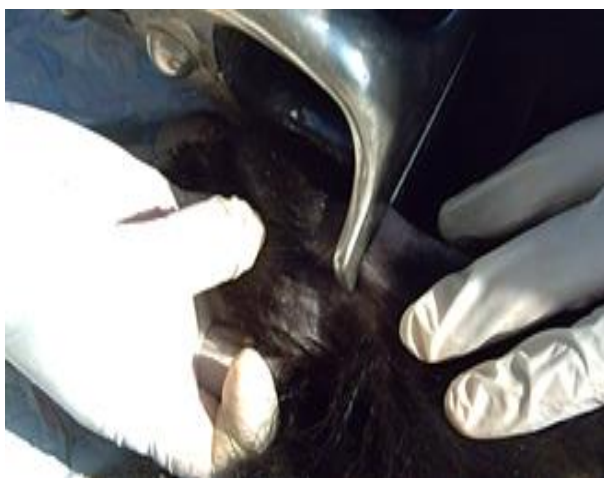


Figure (3): Crushing the spermatic cord by Burdizzo.

Results

The body weight of adult animals were increased significantly ($P < 0.01$) in all castrated groups after the castration. The means of body weight gained of U. torsion and Burdizzo were 7.69 ± 0.26 , 6.12 ± 0.4140 and 6.46 ± 0.419 kg respectively,

while the body weight gained of control group 3.48 ± 0.4283 kg Table (1). Animals in U. torsion group got the lesser body weight gained 6.12 ± 0.414 kg, although these changes were non-significant among the castrated groups.

Table (1): Body weights of adult bucks before and after treatment and weight gained (mean \pm SE). (n=5)

Groups	Body Weight (kg)		Weight Gain (kg)
	Before Treatment	After Treatment	Total
Control	31.88 ± 0.4236 aA	35.36 ± 0.7061 aA	3.48 ± 0.4283 a
U. Torsion	32.02 ± 0.5073 aA	37.78 ± 0.6974 cB	5.76 ± 0.4140 c
Burdizzo	32.18 ± 0.7996 aA	38.64 ± 0.4130 bcB	6.46 ± 0.4190 bc

Different small letters denote to a significance difference ($P < 0.01$) in the one column.

Different capital letters denote to a significance difference ($P < 0.01$) in the one row.

Different small letters in weight gain column denote to a significance difference ($P < 0.01$).

Testicular weight

There were a significant decrease ($P < 0.01$) in the weight of both left and right testes in Burdizzo and right testis of U. torsion groups than the control group.

Whereas the testicular weight of the left testis of U. torsion group remained higher with no significant difference ($P > 0.05$) compared with control group Table (2).

Table (2): Testicular weight (gm) of adult bucks after two months from treatment (means \pm SE) (n=5) demonstrate significant decrease in weight of castrated testes.

Groups	Testicular Weight (gm)	
	Left	Right
Control	134.22 ± 1.7676 a	132.84 ± 0.9368 a
U. Torsion	134.82 ± 1.8274 a	30.42 ± 0.2354 b
Burdizzo	31.42 ± 0.2746 b	30.64 ± 0.3108 b

Different small letters denote a significance difference ($P < 0.01$).

Testicular length

There was a significant decrease ($P < 0.01$) in the length of the both left and right testes of Burdizzo and the right testis of U. torsion

than the control group. In the U. torsion group there were obvious changes in the testicular length Figure (4).

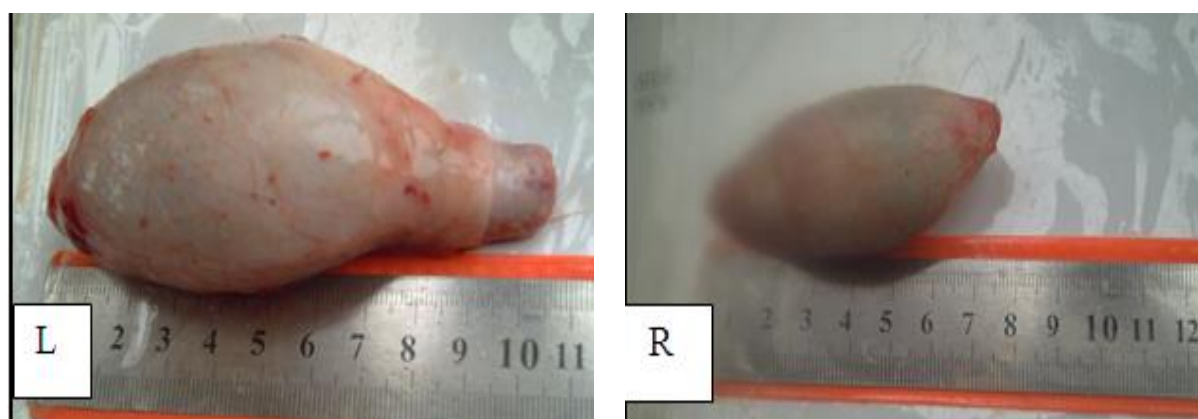


Figure (4): Left and right testes of the U. torsion group of buck.

Whereas there was no significant difference ($P>0.05$) of left testes between U. torsion and control groups Table (3).

Table (3): Testicular length (cm) of adult bucks after two months from treatment (means \pm SE) (n=5) demonstrate significant decrease in length of treated testicles.

Groups	Testicular Length (cm)	
	Left	Right
Control	12.34 \pm 0.2786 a	10.98 \pm 0.1068 a
U. Torsion	12.4 \pm 0.3050 a	7.82 \pm 0.3865 b
Burdizzo	7.98 \pm 0.2615 b	7.68 \pm 0.3247 b

Different small letters denote a significance difference ($P<0.01$).

Testicular circumference

The testicular circumference of both (right and left) testes in Burdizzo and the right testis of U. torsion were significantly decreased ($P<0.01$) compared with the

control group. Whereas no significant difference between the circumference of the left testis of U. torsion and control group Table (4).

Table (4): Testicular circumference (cm) of adult bucks after two months from treatment (means \pm SE) (n=5) demonstrate a significant decrease in testicular circumference of treated testicles

Groups	Testicular Circumference (cm)	
	Left	Right
Control	14.24 \pm 0.3203 a	13.96 \pm 0.3881 a
U. Torsion	14.68 \pm 0.2835 a	8.62 \pm 0.4164 b
Burdizzo	9.34 \pm 0.44 b	8.66 \pm 0.5144 b

Different small letters denote a significance difference ($P<0.01$).

Testosterone hormone

The serum testosterone hormone level in all groups before castration ranged between (0.88 \pm 0.0247 to 0.928 \pm 0.02083 ng/ml). Table (5). After two months of castration the level of hormone shows a significant decrease ($P<0.01$) in all castrated groups than the control, although the level in U. torsion

group significantly higher than the other castrated group, and then the reading after complete orchietomy in the same group. The hormone level after two weeks of complete orchietomy ranged between (0.05 \pm 0.00244 to 0.05 \pm 0.00707 ng/ml) and showed no a significant difference ($P>0.05$) among all groups. Table (7), and Figure (1).

Table (5): Testosterone hormone of adult bucks before treatment (Th.1), two months after treatment (Th.2) and two weeks later from orchietomy (Th.3) (means ± SE) (n=5).

Groups	Testosterone Hormone (ng/ml)		
	Th. 1	Th. 2	Th. 3
Control	0.88±0.0247 aA	0.89±0.02025 aA	0.05±0.007071 aB
U. Torsion	0.928±0.02083 aA	0.2860±0.02839 cB	0.064±0.002449 aB
Burdizzo	0.9240±0.01631aA	0.07±0.003162 bB	0.05±0.004472 aB

Different small letters denote to a significance difference (P<0.01) in same row.

Different capital letters denote to a significance difference (P<0.01) in same column.

Semen assessment

The mean of the semen evaluations (volume, concentration, viability and sperm motility) in all 25 adult animals of all groups before and after two months from treatments was recorded in Table (6).

Table (6): Semen assessments of adult bucks before and after two months from treatment of each group (means ± SE) (n=5).

Groups		Volume (ml)	Concentration (x10 ⁹)	Viability (%)	Sperm motility (%)	
					Individual	Mass
Control	Before	0.554±0.0345	1.96±0.2462	69.54	69.6	68.2
	After	0.588±0.02035 a	1.9±0.2121 a	67 a	69.28 a	69.98 a
U. Torsion	Before	0.6020±0.01985	1.86±0.1364	68.56	68.8	66
	After	0.174±0.009274 b	0.624±0.04377c	22.02c	22.68c	22.58c
Burdizzo	Before	0.568±0.01356	1.7±0.1517	69.52	66.8	66.2
	After	0.136±0.005099d	0.00±0.00 b	0.00 b	0.00 b	0.00 b

There are no significant difference (p>0.05) among before treatments parameters of bucks.

Different small letters denote to a significance difference (P<0.01) among after treatments.

The volume of the semen, the concentration, the viability and sperm motility were seen decreased significantly (P<0.01) in all castrated groups than the control group, although these reading seen significantly higher in U. torsion group than other castrated group.

Pathological changes

Macroscopically evidence

The testes of all treated groups were atrophied and declined significantly (P<0.01) in measurements when compared with the control group, although in the unilateral spermatic cord torsion group the non-treated testes were smaller than the control but greater than the other treated groups.

Histopathological findings

Similar histopathological changes represented with severe damages of testicular tissue were seen in all treated testes in all groups, while the non-treated contralateral testis (left) in U. torsion group showed mild

changes. The changes in the treated testis of the U. torsion group (right) were included severe necrosis of seminiferous tubules, necrosis of leydig cells and increased interstitial space Figure (5), while the untreated testis of the U. torsion group (left) demonstrated mild degeneration in seminiferous tubules with sloughing of spermatids to the center of the duct and absence of leydig cells while Spermatogonia and Sertoli cell are still present Figure (6). The necrosis also included interstitial cells (cell of Leydig) and Sertoli cells besides the spermatogoneum, spermatocyte and spermatids with absence of tubular duct, all these changes happened in Burdizzo group Figure (7). Cross section of testes proper in the control group of the adult buck demonstrating seminiferous tubules, spermatogoneum, Sertoli cells, Spermatocyte, Spermatide, Leydig cells Figure (8).

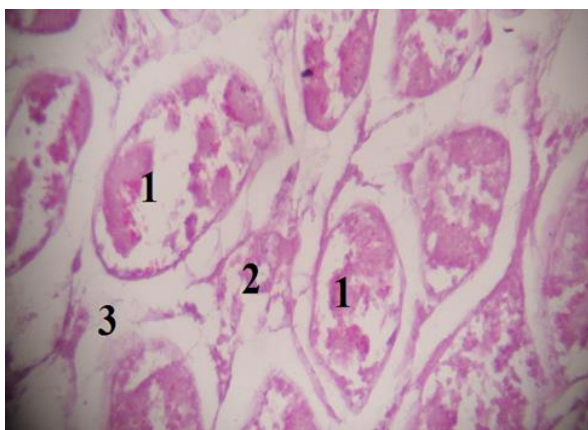


Figure (5): Cross section of testes proper in an adult buck (U. torsion group right testis) demonstrating severe necrosis of seminiferous tubules (1), necrosis of Leydig cell (2) and increased interstitial space (3). H&E, 100X.

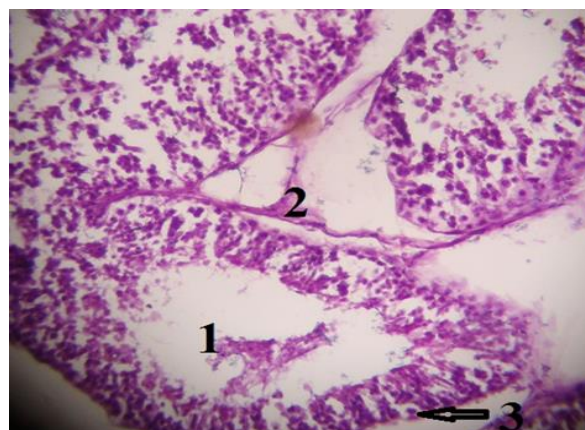


Figure (6): Cross section of testes proper in an adult buck (U. torsion group left testis) demonstrating mild degeneration in seminiferous tubules with sloughing of spermatids to the center of the duct (1) and absence of Leydig cell (2) while Spermatogonia and Sertoli cell are still present (3). H&E, 400X.

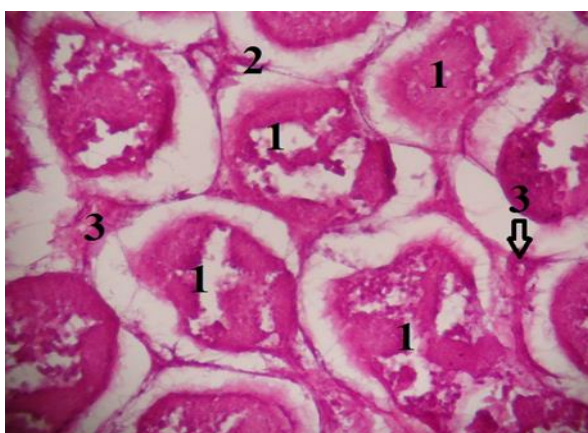


Figure (7): Cross section of testes proper in an adult buck (Burdizzo group) demonstrating seminiferous tubules, necrosis of Spermatogonia, spermatocytes and spermatids and Sertoli cells (1) and absence of tubular ducts, necrosis of Leydig cells (2 and 3) with edema . H&E stain, 100X.

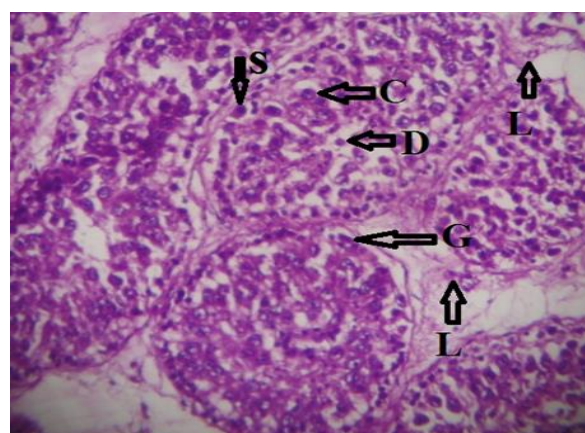


Figure (8): Cross section of testes proper in an adult buck (control group) demonstrating seminiferous tubules, (G) spermatogoneum, (S) Sertoli cells, (C) Spermatocyte, (D) Spermatide, (L) Leydig cell. H& E, 100X.

Discussion

The castration of adult animals by unilateral torsion and crushing with Burdizzo in the present study scored a significant increment in weight gain than the control group. This may have resulted from the castration, which is important for better fat deposition in carcass and for body weight gain improvement. This accords with (6) found that castration in goats could cause increment in body weight gain. Testicular mensuration revealed that the weight, length, and diameter of the testes subjected to the treatments (torsion and bluntly crushing by

Burdizzo) recorded a significant decrease ($P < 0.01$) than the control group. These changes resulted from atrophy and the small size of testes due to the decrease of the blood flow to the testes. This was compatible with (18) found significant atrophied changes in the testes after the ligation of the spermatic cord. The weight, length, and diameter of the testes of the bucks subjected to the treatments (torsion and crushing) recorded a significant decrease ($P < 0.01$) than the control group. The decline in the value of testosterone hormone in the sera of adult bucks before

castration ranged from 0.88-0.92 ng/ml in all animals of the study were related to the time of the study. This result is upheld by (19) who postulated that the period of study may be done out of breeding season. These results revealed that the value of testosterone hormone in the treatment groups (torsion and crushing of spermatic cord) recorded a significant decrease ($P < 0.01$) from the control and this can be explained by the damage of the testicular cells including the leydig cells which are responsible for the secretion of the testosterone hormone caused by the impairment of the testicular blood supply created by the spermatic cord torsion and crushing, and this concurs with (20), found that the testosterone hormone secreted from leydig cells of farm animals and the values of this hormone can be affected by any damage to the testicular cells. In case of the unilateral torsion groups in bucks, there were relatively high level of serum testosterone hormone values compared with other treatment groups, where reached 0.28 ng/ml. seemingly in this study from our opinion there was no compensatory mechanism occurring in the contralateral testis to replace the damage, which occurred in the affected testis. In addition, the damage of the one testicle will result in degeneration of both organs. These results companionable with (21, 22, 23). In contrast, the hemicastration cause increase the level of androgen hormones due to the compensatory mechanism. (24). On the other hand, the low levels of testosterone hormone in the sera of all architected animals credited to the cortex of adrenal gland which secretes the testosterone hormone. This was corroborated with (25, 26) declared that testosterone hormone was secreted from the testes as well as in less quantity from the cortex of adrenal gland. The unilateral torsion of the spermatic cord can cause damage to the contralateral testis, some investigators have demonstrated a detrimental contralateral effect of the unilateral testicular torsion (21, 22, 23, 27), other investigators didn't support this

concept (28, 29). The mechanism of the injury to one testis following the contralateral testicular ischemia is also a subject of controversy. Many mechanisms have been proposed to explain the contralateral testicular damage, the auto immunization against Spermatogonia, the free oxygen radical formation after detorsion and the overproduction of nitric oxide by activated inducible nitric oxide synthase. (30, 31). Furthermore, the mechanism by which the contralateral testicular blood flow is affected after the unilateral vas deferens obstruction may due to the vasospasm in the contralateral testis suggested arise by a neurovascular pathway triggered by an ipsilateral testicular stimulus, running through a sympathetic reflex arc, resulting in decreased blood flow (32). The semen evaluation values in adult bucks including the volume, the concentration, the viability the individual and mass motility were similar to what conducted by (33, 19). There was a direct relationship between the serum testosterone level and the total sperms count, viability and motility. The low levels of testosterone associated with low values of semen characteristics and this was found in the present study in agreement with (34) substantiated that ram semen parameters were affected by the testosterone hormone. Certain values of the semen parameters in the unilateral torsion group which raised significantly ($P < 0.01$) than the other treated groups due to the unilateral testicular injury and this was suggested by (35) who found that the unilateral testicular torsion seems to have bilateral abnormalities that result in decreased spermatogenesis from the normal values. It was unclear whether these abnormalities were due to an autoimmune process that occurs after the rupture of the hematotesticular barrier leading to the formation of antisperm antibodies or because of reperfusion-induced injury to the testis. In addition, these results contradicted with (24) who concluded that hemicastration in the goats improved semen characteristics due to

the compensatory mechanism of the remaining testis and these different results of the two researchers may have come from the removal of one testis from the animal while in the present study the treated testis attached in place of the body. All treated testes were observed grossly as smaller in parameters than the control groups and this was due to the complete occlusion of blood and nerve supply to the testes as well as congestion of blood vessels of spermatic cord, which lead to a noticeable atrophy of the testes, and similar results have been demonstrated by (18). Severe suppression of the spermatogenesis, reduced numbers of spermatozoa, no sperms in the seminiferous tubules and no spermatids with depletion of the germ cells and degeneration of leydig and Sertoli cells were seen microscopically. These results were attributed to the obstruction of the blood and nerve supply to the testes by occlusion of spermatic cord and this is consistent with (23) found that obstruction of the spermatic cord result in pathological changes in the testes including degeneration of leydig and Sertoli cells and affection of seminiferous tubules leading to produce seminal plasma only in ejaculate without sperms. The treated testes in the unilateral torsion group had exactly the same changes in the other treated group while the untreated ones (the contralateral) suffered from a mild degeneration with less damage of the interstitium produced by the reduction of the blood flow in the non- twisted cord which gradually increased after the procedure and this agreed with (36), and involved the generation of toxic reactive oxygen species (for example, hydrogen peroxide, hydroxyl radical and superoxide anion) that may damage several cellular components by

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peroxidation of cell membrane lipids and this what suggested by (37). From the results of this experiment, the spermatic cord torsion caused testicular damage in adults. Also the unilateral spermatic cord torsion caused damage to the contralateral testis, two adult bucks from the unilateral torsion group developed hydrocele which may represent an imbalance in the secretory and absorptive capacities of the layers of the tunica vaginalis as a result of an inflammatory reaction. (38) Noticed similar changes who stated that the hydrocele might accompany torsion of the spermatic cord in rams. Many complications after the completion of the castration by all techniques were seen like a noticeable change in the behavior of the animals represented by increase restlessness time, lying on the ground, rolling and kicking of belly or flank. All these concurrent with (39) who declare that castration can cause signs of pain to the affected animals. The common complication for most surgical operations is wound infection and this came from the attack of the wound by pyogenic bacteria from the environment of the affected animals and this agreed with (40). The castration by Burdizzo can cause more severe acute inflammatory responses, in terms of pro-inflammatory cytokine gene expression, in the testis and epididymis. (41). The spermatic cord torsion considers as a simple method for castration in the local Iraqi black goats with no major differences when compared with traditional castration methods, so that this method can be used efficiently as a modern technique for effective castration in goats, furthermore, the unilateral spermatic cord torsion can cause damage to the contralateral testis.

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