Pathological and Biochemical Studies on Chemically Castrated Bucks

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ABSTRACT

Thirty sex apparently healthy Balady bucks, of 1-2 year age were divided into 6 equal groups (gps) .The bucks showed normal libido and ability to serve. Gps 1& 2 were injected intratesticularly (IT) with 20% formalin and 20% glacial acetic acid respectively. Gps 3 & 4 injected intraepidedymally (IE) with 20% formalin and 20% glacial acetic acid, respectively. Gps 5& 6 were the control (IT and IE injected with saline, respectively). Two blood–samples were collected from all animals 30 and 60 day post- injection (PI). One sample (on heparin) was used for hematological study. The other sample was left to clot, and serum was separated for biochemical and hormonal studies. Semen was collected 30 and 60 day PI using the artificial vagina for semen evaluation. Bilateral surgical removal of the testes and epididymidis of three bucks, from each group, was done 30 and 60 day PI, and after blood-collection for pathological studies. The testes and epididymidis were fixed in Bouns solution for pathological studies.

The blood picture was almost normal with a non-significant elevation of the leukocytic count in gps.1-4. The total protein, albumin, AST, ALT and creatinine in gps 1-4 were almost similar to the control. Non- significant changes of the testosterone hormone was noticed in gps.3 and 4, but it was sharply decreased in gps. 1 and 2. Semen was easily collected from gps. 3 and 4 after subsiding of epididymitis and difficult in gps 1and 2, however, all treated groups showed azoospermia. Gps. 3 and 4 showed coagulative necrosis of the epididymal epithelial lining, besides necrosis and calcification of its sperm-content. Fibrosis was seen in gp.4. The seminiferous tubules showed moderate spermatogenesis with prominent sclerosis in gp.2. Gps. 1and 2 showed, testicular degeneration and sperm granuloma, besides focal calcification, which was surrounded by fibrous connective tissue infiltrated with mononuclears. It could be concluded that castration of bucks could be successfully performed by formalin and glacial acetic acid-inoculation in the testes and epididymides.

INTRODUCTION

Nowadays the breeding of goats is widely spread allover the world because their easy management and feeding. They are considered a good source of meat and milk, thus their economic value is high. Castration of male domestic animals by open surgery frequently requires post-operative care to minimize the risk of hemorrhage and infection. Efforts to find chemical materials that sclerose the testis were started with the American pig farmers to reduce the bad taste, induced in adult boar-meat by androgen *(1)*. Chemical and immunological male-sterilization were previously tried in male cattle *(2)*, ram *(3)*, buck *(4)* and rat *(5,6)*. Intratesticular injection of danazol *(7)*, glycerol *(8,9)*, glucose and sodium chloride *(10)*, zinc arginine *(11)*, formalin *(12)*, calcium chloride *(6,13)*, potassium permanganate and glacial acetic acid *(14)*, were used. Moreover the intraepididymal injection of calcium chloride *(15)*, 1.5 % chlorhexidine digluconate *(16)*, ethanolamine in dimethylsulphoxide (17) and zinc arginine *(11)* were also tried. Some of the previous chemicals caused permanent sterility by damaging both the seminiferous tubules and interstitial tissues *(5)*. Many complications followed the use of these chemicals, thus a complete effective chemosterilizing agent was not identified *(4,18)*.

The purpose of this work was to evaluate the efficacy of the intratesticular and intraepididymal injection of either 20%

formalin or 20% glacial acetic acid as chemosterilizing agents for bucks to optimize the most effective one for further application on a large scale program.

MATERIAL AND METHODS

Thirty- sex apparently healthy balady bucks, of 1-2 year age with normal libido and ability to serve were collected from different flocks in Sharkia governorate. Bucks were divided into 6 equal groups. Under complete aseptic conditions, gps. 1 and 2 were intratesticularly (IT) injected with 2 ml of 20% formalin gp.1 and 2 ml of 20% glacial acetic acid gp2. Grs 3 and 4 were intraepididimally (IE) injected with 1 ml of 20% formalin (gp.3) and 1 ml of 20% glacial acetic acid gp.4. Gps. 5 and 6 were the control as gp.5 was IT injected with 2 ml saline, and gp.(6) IE injected with 1 ml saline. Two blood- samples were collected from the jugular vein at 30 and 60 day post injection (PI). One sample was collected on heparin as anticoagulant for hematological studies *(19)* and the other one was left to clot and centrifuged at 3000 rpm for 10 minutes to separate serum for the determination of serum-total-proteins *(20),* albumin *(21),* serum AST and ALT *(22),* creatinine *(23)* and testosterone hormone *(24).* Semen was collected 30 and 60 day PI using the artificial vagina for semen collection and evaluation to determine the efficacy of this type of castration. Bilateral testicular and epididymal surgical removal was done for 3 bucks from each group, 30 day PI, and for the other 3 bucks, 60 day PI after blood-collection. Surgical castration, for pathological study, was done under local anesthesia (10 ml procaine hydrochloride) at the neck of the scrotum *(25)* and under aseptic condition. The testes and epididymidis were fixed in Boun`s solution and embedded in paraffin wax. Five micron paraffin sections were prepared, stained with hematoxylin & eosin and examined microscopically *(26).* The obtained data were tabulated and statistically analyzed *(27).*

RESULTS AND DISCUSSION

Chemical castration using 20% formalin or 20% glacial acetic acid, either intratesticularly or intraepididymally induced severe pain, manifested by elevating of the hind-limb with much discomfort and grunting on touching the testis. This pain with some degree of loss of appetite continued for 4-5 days with formalin and for 4-8 days with the glacial acetic acid. The injected testes were inflamed and edematous with an obvious swelling in the gps.1 and 2 . Gps. 3 and 4 showed epididymal swelling which extended to involve the whole spermatic cord. Similar testicular swelling was induced by IT injection of balanced zinc solution and calcium chloride *(4,28).* Both formalin and glacial acetic acid induced inflammation when injected either IT or IE. The inflammation was manifested by pain and swelling. It is believed that the initial testicular swelling is due to edema *(4)* and could be ascribed to an immediate inflammatory tissue responses induced by the treatment *(29).* The injected testes underwent some atrophy on the 30th day PI which could be attributed to the subsiding inflammatory signs together with the pressure atrophy of the seminiferous tubules due to the blockage of sperm transport in case of IE injection *(11, 30).* The testicular atrophy in gps. 1 and 2 may be due to degeneration and necrosis of the testicular tissue and/or fibrosis and retraction later on *(30).* Such lesions led to atrophy of the testicular parenchyma *(4).* However, the IT injection of bulls with calcium chloride showed no marked alteration inspite of the reported testicular atrophy on the 60th day after IT ethanol treatment *(18).*

Two undefined stages were recorded after IE or IT injection of the chemosterilizing agent. Stage,1 (10-20 day PI) in which the testes enlarged, become painful, hard and did not move easily inside the scrotum. Stage,2 (30–60 day PI) in which the testes were flabby, slightly atrophic, and freely moved inside the scrotum.

Macroscopically, the testes of gps. 1 and 2) were edematous from the first day PI and remained enlarged till the 30th PI (Fig.1). The Testes of gp.2 were more painful to touch. The testicular parenchyma was soft and flabby, 30 day PI. The testicles became small in size and firm in consistency but the epididymides were

normal, 60 day PI. Gps. 3 and 4 showed markedly swollen epididymides on the 30th day PI. The epididymides became reduced in size and firm in consistency 60 day PI with atrophied testis. The injection of the previous materials (IT or IE) resulted in swollen testes by inflammatory edema *(4)* and destruction of the seminiferous tubules, releasing the germ-cells outside the seminiferous tubules. The released germ-cells became exposed to the body-immune-system. The later consider such germ-cells foreign antigen which is phagocytized by the antigen presenting cells, initiating cellular-immunity in the form of sperm-granuloma *(17)*. Sixty day after IE injection, the inflammation subsided ending with testicular atrophy and blocking of the seminiferous tubules *(11,30)*. The testicular atrophy, in case of IT injection, may be due to the degeneration, necrosis and sclerosing of the testicular tissue *(29)*.

Microscopically, 30 day after IE injections with formalin gp.3 or glacial acetic acid gp.4, hyperplasia of the epithelial linings of the epidedymal tubule was seen with necrosis and desquamation of the necrotic cells inside the epididymal tubular lumen with interstitial fibrous connective tissue proliferation (Fig.2). Hemosiderosis was seen in the sclerotic interstitial tissue, particularly with the glacial acetic acid injection (Fig.3). The epididymal tubules were empty of sperms and others contained necrotic debris (Fig.4). Cystic dilatation of the epidedymal tubules was seen with necrotic debris (Fig.5). The interstitial tissue was infiltrated with round cells. Testicular edema was seen (Fig.6). Leukocytic infiltration in the interstitial tissue is due to tissue damage and released chemotactic factors *(10)*. The Leydig cells were apparently normal. Coagulative necrosis of the epidedymal cell-linings and necrotic debris in the tubular lumen were detected (Fig.7). Our results are in a partial agreement with others *(3)*, they mentioned that formalin resulted in coagulative necrosis when injected intraepididymally and intratesticularly in rams.

Coagulative necrosis of the epididymal epithelial lining with spermatostasis and calcified necrotic sperms were observed on the 60th day after IE injections (Fig.8). The seminiferous tubules showed moderate spermatogenesis (Fig. 9) .The lesions were severe in gp.2 with prominent fibrosis. Congested blood vessels and edema among the seminiferous tubules gps.1 and 2 with moderate spermatogenesis, were seen 30 day after intratesticular injection. Testicular degeneration was characterized by vacuolated germ-cells and aspematogenesis (Fig.10). The basement membrane of the seminiferous tubules was thickened with loss of germ-cells (Fig.11). Focal calcification, surrounded by fibrous connective tissue infiltrated with mononuclears was detected (Fig. 12). Vacuoles are dilated smooth and rough endoplasmic reticulum of the Sertoli cells *(31)*. On the other hand, others *(25)* described the vacuoles as damaged Sertoli cells, induced by the injected chemicals. Such damage may be attributed to the accumulation of sperms and fluid which led to increased hydrostatic pressure proximal to the site of injection *(32)*. Aspermatogenesis, characterized by numerous spermatozoids (adjoining the basement membrane), few spermatocytes and no mature spermatozoa was seen (Fig.13).

Sixty day after Intratesticular injection, coagulative necrosis of the seminiferous tubules was seen. Some cases, particularly, in gp.2, showed necrosis and calcification of the seminiferous tubules and Leydig cells (Fig.14). Testicular calcification was seen (Fig.15). Sperm-granuloma was characterized by fibrous connective tissue infiltrated with mononuclears and giant cells and calcification (Fig.16). The granulomatous reaction may be due to the escape of the spermatids and spermatozoa from ruptured tubules to be exposed to the immune system. The germ-cells exposure to the immune system initiates a local immunological response against the exposed spermatids and spermatozoa *(17)*. Hyalinized seminiferous tubules surrounded by necrotic Leydig cells were seen.

A significantly elevated leukocytic-count was found 30 day after IT or IE injection gps.1-4 and returned to the normal level 60 day PI (Table 1). The leuckocytosis may be

attributed to general defense, besides the local inflammatory reaction at the site of injection.

The biochemical studies, concerning both the liver and kidney function tests (Total protein, Albumin, AST, ALT and creatinine) showed non significant changes between the control and each of the treated groups (Table 2). This may be attributed to the local degenerative and destructive affects of the injected chemical which consequently limited or prevented the absorption of these chemicals into the circulation. Moreover, the injected dose was too small to produce an adverse systemic side effect. No available data were found, concerning the liver or kidney function tests in case of chemically castrated animals.

The libido of the IE chemically sterilized bucks was reduced for 5-7 day PI in gps. 2 and 4. This may be due to the painful status of bucks. However, few days were enough for libido to return to its nearly normal level which may be due to subsiding of the inflammatory signs. This phenomenon was confirmed by the obtained normal level of testosterone hormone as well as the recorded normal Leydig cells in gps. 2,4,5 and 6 30 and 60 day PI. Our result agrees with previous workers ***(11)*** who recorded normal testosterone level after IE zinc arginine injection in dogs. However, others ***(15)*** reported a normal mounting time without measuring the testosterone level after IE calcium chloride injection of rams. Meanwhile, other studies showed a reduced testosterone level in pigs after IE injection of zinc acetate or calcium acetate ***(33)***. This variation may be attributed to the ability of the injected material to destroy the Leydig cells. The dose, concentration and the species of animal may play a role. On the other hand, the libido of gps.1 and 2 showed a significant decline on the 30^{th} and 60^{th} day PI which was manifested by a prolonged mounting time (Table 3). This was emphasized by the reduced testosterone level (Table 3) and the necrotic Leydig cells. Similar results were recorded after 30 day of IT injection of calcium chloride to rats ***(6)***, goats ***(4)***, and was also recorded after IT zinc arginine, zinc acetate or calcium acetate injection in pigs ***(33)*** and IT calcium chloride injection in bulls ***(34)***. Microscopically, the testes showed necrosis and complete fibrosis of the Leydig cells of rats, bucks and dogs after IT injection of different sterilizing agents ***(29, 35,28*** respectively). These findings are in agreement with ours. The present data are inconsistent with those ***(36)*** who found that the IT injection of 70% glycerol in dogs did not result in azoospermia or sterility. A normal testosterone-level with normal semen picture was reported after IT calcium chloride injection in bulls ***(18)***. The reported low level of testosterone after formalin and glacial acetic acid IT injection was the result of degeneration in the interstitial Leydig cells. The efficacy of formalin and glacial acetic acid in inducing chemical castration was supported by the necrosis of the seminiferous tubules and interstitial cells along with the significant fibrosis ***(4)***.

Semen collection was easy after subsiding of orchitis gps.1 and 2 and epididymitis gps.3 and 4. Fifteen day PI, semen collection was possible due to the normal libido and normal testosterone-level. Meanwhile, the collected semen showed azoospermia which may be attributed to the damaged testicular parenchyma and the occluded epididymal duct. All the ejaculates were characterized by complete absence of motility with head-tail-fragmentation. Similar results were previously recorded ***(15)***. Meanwhile, semen collection, from gps. 1 and 2 was difficult due to the damaged testicular parenchyma and the reduced testosterone level. After many trials, semen was collected to show azoospermia with necrotic debris. Similar findings were reported ***(7)***. Azoospermia was observed in this work and previous investigation in boar, dog, rat and ram ***(3,5, 37, 38,*** respectively). Normal semen was collected after IT injection of calcium chloride in bulls ***(18)*** or 70% glycerol in dogs ***(36)***. Other investigators did not evaluate semen but their histopathological study revealed complete testicular fibrosis ***(28),*** total necrosis and fibrosis ***(4,29,35)*** and destruction of the seminiferous tubules ***(39)***

It could be concluded that chemical castration, by IT or IE inoculation of formalin or glacial acetic acid, particularly IT, was save and economical for the sterilization of bucks.

Table 1. Blood picture of the control and chemically castrated bucks , 30 and 60 days PI (n = 6)

Time in days PI	Site of injection	Groups	Blood parameters			
			RBCs (10^6/ul)	Hb (%)	PCV	WBCs (10^3/ul)
30	IT	C	13.383±0.344	7.62±1.33	30.33±0.76	5.966±0.206a
		F	13.391±0.400	8.83±0.09	29.00±1.48	6.918±0.183bc
		G	13.140±0.280	8.87±0.13	28.83±0.48	7.158±0.553c
	IE	C	12.935±0.332	8.50±0.19	28.50±1.09	5.911±0.199a
		F	13.491±0.385	8.98±0.12	29.50±1.06	6.836±0.90bc
		G	13.038±0.345	8.87±0.16	30.17±0.95	6.895±0.150bc
60	IT	C	13.350±0.491	8.78±0.27	30.67±0.99	6.093±0.156a
		F	13.350±0.387	8.65±0.19	29.50±0.92	6.520±0.261abc
		G	12.966±0.466	8.43±0.17	29.83±1.30	6.276±0.201ab
	IE	C	13.011±0.438	8.48±0.12	29.33±0.80	5.911±0.150a
		F	12.871±0.558	8.60±0.18	30.00±1.24	6.200±0.135ab
		G	12.798±0.553	8.35±0.18	29.17±1.11	6.265±0.123ab

C=control, F=formalin, G=glacial acetic acid

Values with different stars (between control & treated group) within the same item, the same column & the same period differed significantly at least at <0.05

Table 2. Some biochemical parameters of the control and chemically castrated bucks at 30 and 60 days PI (n = 6)

Time in days PI	Site of injection	Gps	Biochemical parameters				
			Total protein (gm/dl)	Albumen (gm/dl)	AST (i/u)	ALT (i/u)	Creatinine (mg/dl)
30	IT	C	6.28±0.11	3.00±0.08	25.50±1.23	3.33±0.80	0.81±0.03
		F	6.45±0.15	3.05±0.11	24.50±1.31	4.00±0.77	0.84±0.04
		G	6.37±0.09	3.07±0.05	22.67±1.63	3.83±0.79	0.84±0.03
	IE	C	6.25±0.12	3.02±0.09	21.00±2.85	3.67±0.61	0.86±0.04
		F	6.55±0.15	2.95±0.06	24.17±1.82	3.67±0.61	0.83±0.02
		G	6.37±0.07	3.07±0.06	24.83±1.30	3.67±0.98	0.82±0.03
60	IT	C	6.37±1.00	3.03±0.08	27.67±2.16	5.33±1.05	0.78±0.05
		F	6.48±0.16	3.13±0.12	27.50±2.20	4.83±0.79	0.83±0.05
		G	6.47±0.10	3.20±0.06	26.50±2.80	4.33±0.95	0.78±0.05
	IE	C	6.63±0.11	3.03±0.08	24.50±3.25	5.67±0.61	0.77±0.05
		F	6.40±0.09	3.07±0.06	28.50±2.63	4.83±0.87	0.80±0.04
		G	6.43±0.07	3.13±0.07	26.33±2.56	4.83±1.14	0.76±0.03

C=control, F=formalin, G=glacial acetic acid

Values with different stars (between control & treated group) within the same item, the same column & the same period differed significantly at least at <0.05

Table 3. the mounting time and serum testosterone hormone level at 30 days (n = 6) and 60 days (n=3)

Time in days PI	Site of injection	Gps	Mounting time (seconds)	Testosterone hormone (ng/dl)
30	IT	C	12.50±2.04ab	4.32±0.32def
		F	85.33±8.82e	1.78±0.39b
		G	101.60±8.03f	0.97±0.20a
	IE	C	16.50±2.57abc	4.45±0.30ef
		F	29.17±3.12bc	3.96±0.15cde
		G	28.17±4.01bc	3.86±0.23cde
60	IT	C	12.83±2.65ab	5.06±0.24f
		F	49.00±5.92d	1.48±0.22ab
		G	108.33±11.16f	0.89±0.13a
	IE	C	9.33±1.38a	5.18±0.22f
		F	31.67±4.94c	3.58±0.20cd
		G	34.17±4.08cd	3.32±0.18c

C=control, F=formalin, G=glacial acetic acid.

Values with different stars (between control & treated group) within the same item, the same column & the same period differed significantly at least at <0.05

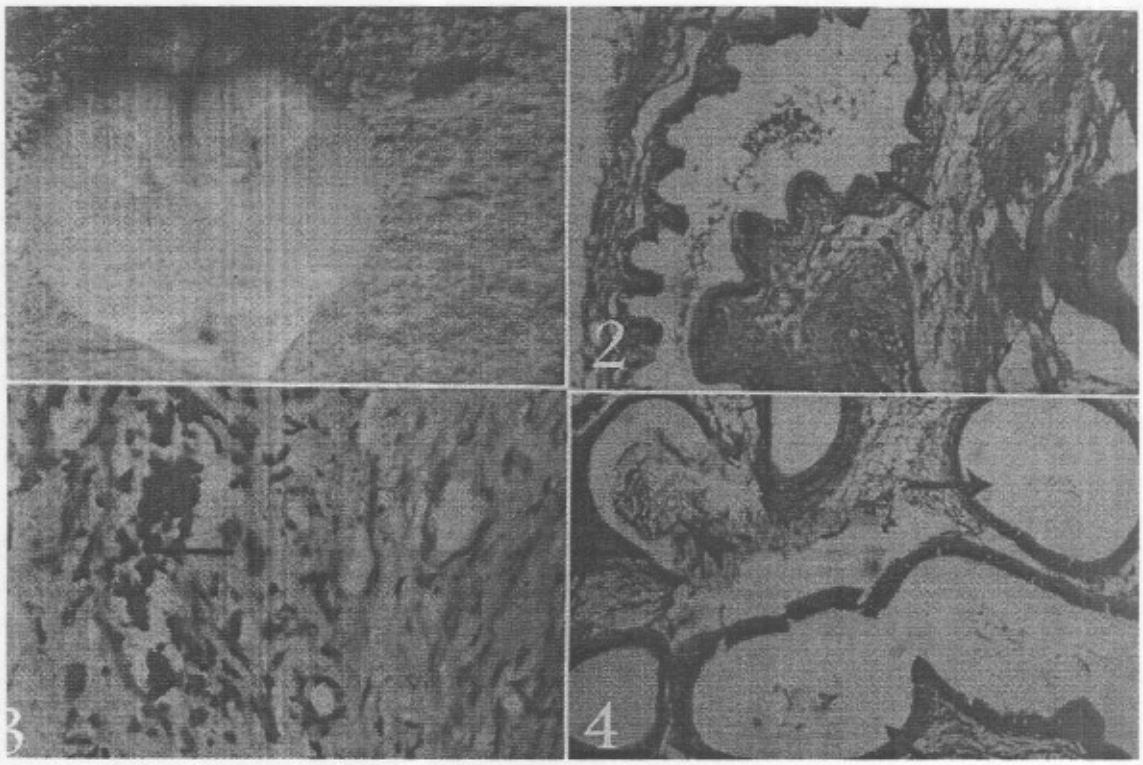

Fig.1: GP.(2),testis,30day PI, the testes are enlarged and edematous.

Fig.2:GPS.(3&4), 30 day PI,epididymis showing, hyperplasia of the epithelial linings of the epididymal tubule and interstitial tissue proliferation H&E, x600

Fig.3: GPS.(3&4), 30day PI, epididymis showing ,hemosiderosis and sclerosis . H&E , x 600

Fig.4: GPS.(3&4), 30day PI, Empty epididymal duct except of necrotic debris . H&,E x 600.

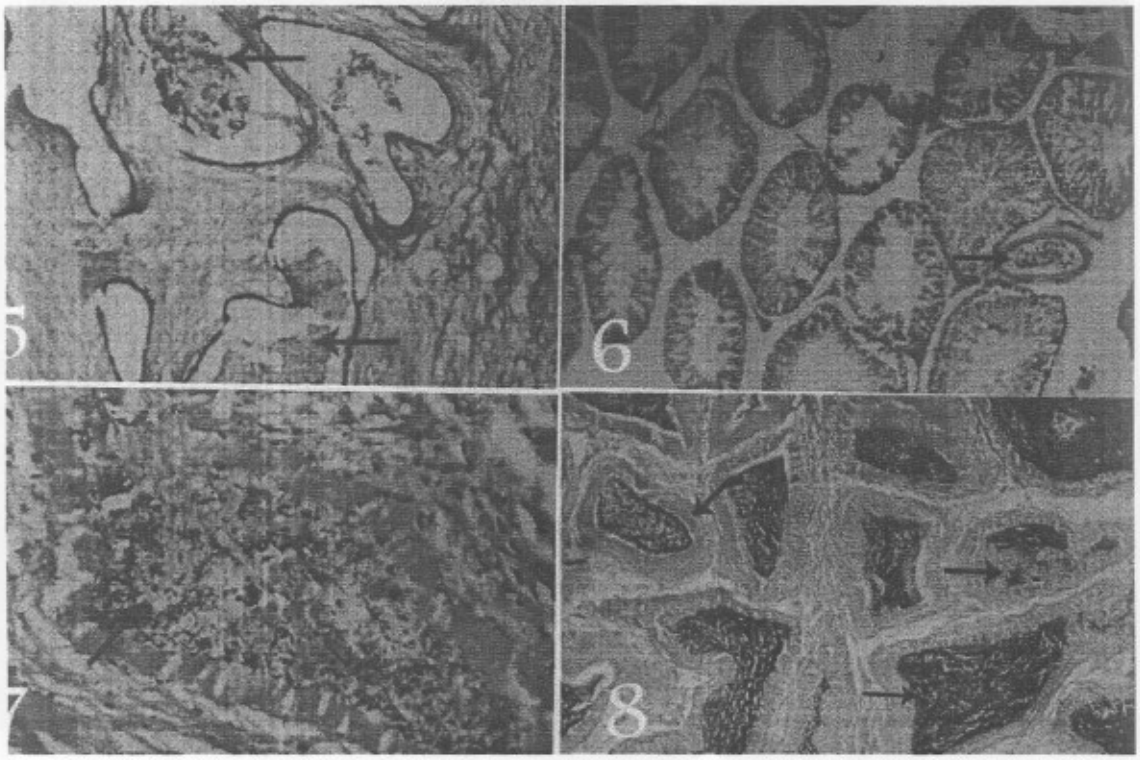

Fig.5: Gps.(3&4), 30day PI, epididymis showing ,cystic dilatation of the epidedymal duct with necrotic debris.H&E, x300
Fig.6: Gps.(3&4), 30day PI, testicular edema. H&E, x300
Fig.7: Gps.(3&4), 30day PI, epididymis showing, coagulative necrosis of the ductal cell- linings and necrotic debris in lumen .H&E, x600
Fig.8:Gp.(4),60 day PI, epididymis showing, coagulative necrosis of the epithelial lining with spermatostasis and calcification . H&E, x300

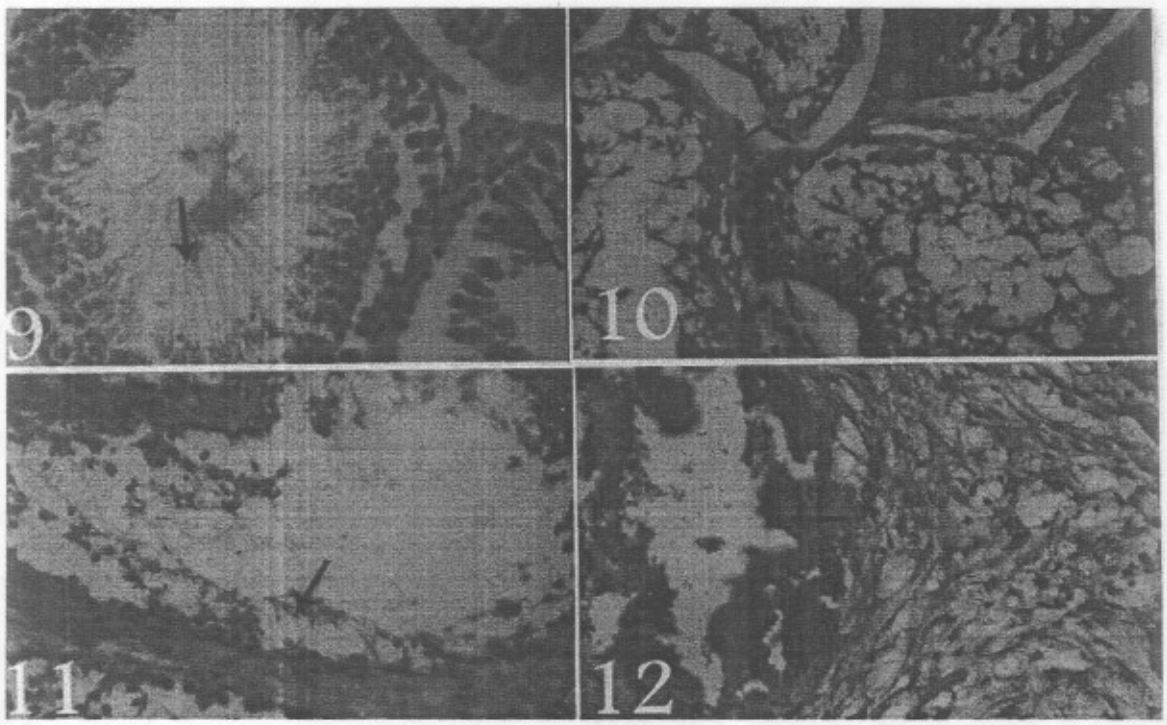

Fig.9: Gp.(4),60 day PI, testis showing, moderate spermatogenesis(arrow) .H&E x1200.
Fig10: Gps.(1&2),30 day PI testis showing, vacuolated germ-cells with aspermatogenesis .H&E, x1200
Fig.11: Gps.(1&2),30 day PI, testis showing, thicked basement membrane of the semineferous tubule with loss of the germ-cells (arrow).H&E, x1200
Fig.12: Gps.(1&2),30 day PI, testis showing, focal calcification, surrounded by fibrous connective tissue infiltrated with mononuclears. H&E x600

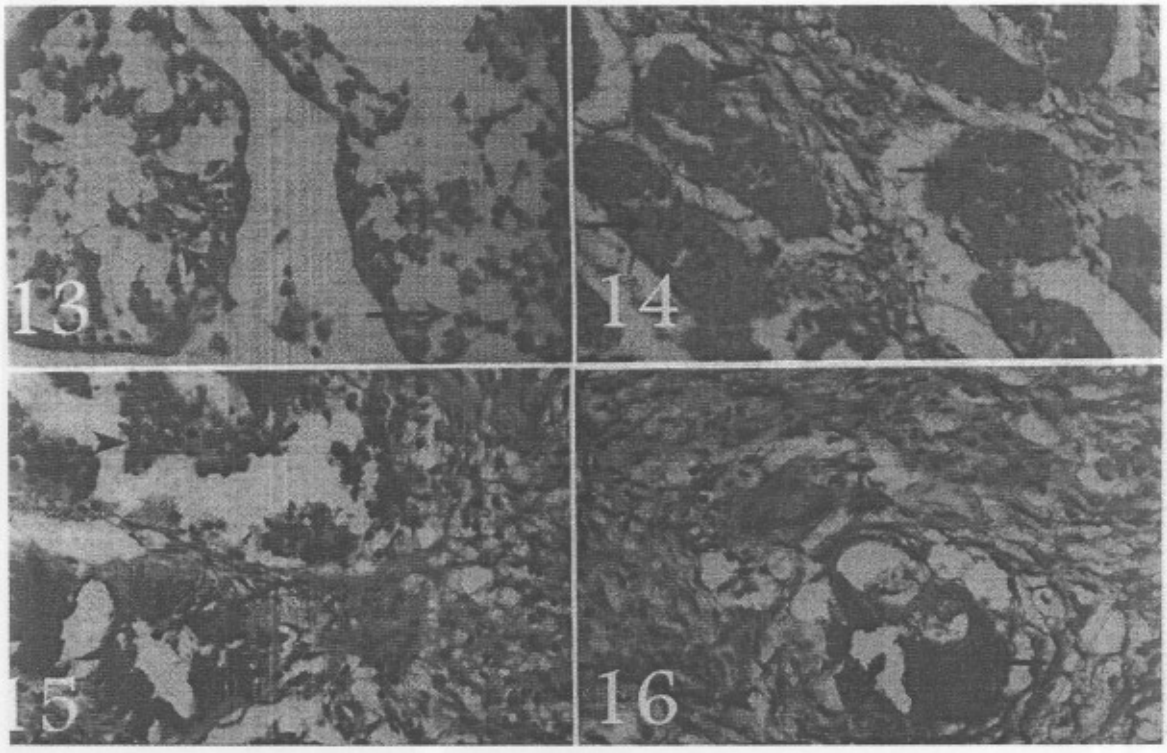

Fig.13: Gps.(1&2),30 day PI, testis showing, aspermatogenesis, characterized by numerous spermatozoids joining the basement membrane, few spermatocytes and no mature spermatozoa. H&E, x600.

Fig.14:Gp.(1),60 day PI, testis showing, necrosis and calcification of the seminiferous tubules with necrotic Lyding cells . H&E x600.

Fig.15: Gp.(1),60 day PI, testis showing, calcification of the semineferous tubules.H&E, x600.

Fig.16: Gp.(1),60 day PI, testis showing, sperm- granuloma characterized by focal calcification surrounded by fibrous connective tissue infiltrated with mononuclears and giant cells. H&E x600.

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الملخص العربى

دراسات باثولوجيه وبيوكيميائيه على الجديان المخصية كيميائياً

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تم إجراء هذا البحث التطبيقى على عدد ٣٦ من ذكور الماعزالبلدى البالغه عن عمر ١-٢ سنه وقسمت الى ٦ مجاميع متساوية. المجموعتين الاولى والثانيه تم حقنها داخل الخصية ٢سم^{3} فورمالين ٢٠% و٢سم^{3} حمض الخليك الثلجى على التوالي .والمجموعتين الثالثه والرابعه تم حقنها بالجرعات السابقه داخل البربخ على التوالي .المجموعتين الخامسه والسادسه تركتا كضابط للتجربه وتم حقنها بمحلول ملحى داخل الخصيه والبربخ على التوالي. وتم الحصول على الخصيه والبربخ جراحيا بعد ٣٠ و٦٠ يوم من الحقن للفحص الباثولوجى مع عينات دم للفحص الكيميائي. وقد تم تسجيل التغيرات الظاهريه على الخصية والبربخ وكانت عباره عن ورم أوديمى مع ألم عند الملامسة إختفى بعد ٤-٨ ايام من الحقن. بالنسبة للفحص الكيميائي فقد أظهرت الدراسة عدم وجود أى تغيرات فى البروتين الكلى والزلال وأنزيم ألانين أمينو ترانسفيريز (ALT) وأسبرتيت أمينو ترانسفيريز (AST) والكرياتينين. فى حين أن صوره الدم أظهرت زيادة غير معنوية فى عدد كرات الدم البيضاء . ووجد أن الرغبة الجنسية ومستوى هرمون التستسترون كانا فى مستوى اقرب الى الطبيعى فى حاله الحقن بالبربخ فى حين سجلا انخفاضا كبيرا فى حالة الحقن داخل الخصية. وقد أدى الحقن بالخصية أو بالبربخ الى تدهور شديد فى معايير السائل المنوى. وكانت التغيرات الهستوباثولوجيه عباره عن استحالات وتنكرز وتكلس للخلايا المبطنه لأنابيب الخصية والبربخ وخلايا السرتولى مع إختفاء الحيوانات المنوية الحية وحدوث تنكرز وتكلس بها مع تليف النسيج بين الانابيب واختفاء الخلايا بين القنوات المنويه .وكانت حده التغيرات اشد فى حاله الحقن بحامض الخليك الثلجى .

وأثبتت الدراسة مدى جدوى الخصى الكيميائى وبخاصه الحقن داخل الخصيه بالفورمالين و حامض الخليك الثلجى وذلك لتجنب مخاطر الخصى الجراحى