

## **EFFECT OF VITAMIN C ON APOPTOTIC GERM CELLS OF CRYPTORCHID TESTIS IN RABBITS**

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### **ABSTRACT**

The study examined the effect of vitamin C on apoptotic testicular germ cells in experimentally induced cryptorchidism in the male rabbits. Oral administration of vitamin C (10 mg. kg body weight) for 8 weeks to rabbits showed significant elevation in testis parameters in treated cryptorchid groups (TC), also significantly elevated the number of germ cells compared to untreated cryptorchid groups (CC). However, vitamin C caused significant depression in apoptotic germ cells and apoptotic tubules in treated cryptorchid groups (TC) compared to untreated cryptorchid groups (CC). Vitamin C alleviated the deleterious effect of oxidative stress in cryptorchidism.

### **INTRODUCTION**

Cryptorchidism is a well known as clinical condition associated with male infertility. The word crypt orchid literally means a testis is hidden from view and appear failure of the descend in to the scrotum(1). The two distinct mechanism for cell death, the necrosis and apoptosis. They differ from each other in their etiological and cytological characteristic. The necrosis is mostly result from a major cell insult such as that caused by mechanical, ischemic, or toxic damage. In contrast apoptosis is occurs as response to less sever injury and involved in the development and remodeling of normal tissue(2)

The apoptosis is a mechanism by which the body maintains cellular balance in physiological processes, this is delicately maintained and any disturbance in its control results in disease(3).During the apoptosis the chromatin showed condensation take place(4) which results in a free -OH group at the 3' end of the deoxyribose sugar of the condensed DNA.

Vitamin C is important chain an breaking antioxidant capacity of the seminal plasma. Dietary supplementation protects human sperm from endogenous oxidative DNA damage, thereby decreasing the risk of genetic defects and particularly in populations with low vitamin c level, such smokers(5). Vitamin C (ascorbic acid) is supported of spermatogenesis at least in part through its capacity to reduce  $\alpha$ -tocopherol and maintain this antioxidant in an active state, also its self maintained in a reduced state by a GSH- dependent dehydro ascorbate reductase , which abundant in the testis(6).Many investigation determined effect of cryptorchidism on testicular apoptosis but to our knowledge there is no polished report on the effect of vitamin C on the cryptorchid testis especially apoptosis by fuelgen reaction.

## **MATERIALS AND METHODS**

Fifteen adult male rabbits of 4-5 month old buing bought from Animal market in Basrah .The animals were divided in to three groups of five rabbits in each group as in Table-1

Table-1- summary of a study design

groups	Name of group	Treatment
Group 1	Normal non crypt orchid(control) ( NCC)	These animals were give distal water for 8 weeks
Group 2	cryTpt orchid( control) (CC)	These animals were rendered unilaterally crypt orchid in the right testis and given distal water for 8 weeks
Group 3	crypt orchid treated (TC)	These animals were rendered unilaterally crypt orchid in the right testis and given vita c(10 mg-kg BW) for 8 weeks

The induction of cryptorchidism (7) was done under stick aseptic conditions by the following procedure :The animals were anaesthetized with ketamin (75 mg per kg B.W)and the right testis was mobilized through transverse inguinal incision and pushed back the abdomen through the internal inguinal ring which was sub sequently closed with chromic sutures. All the animals sub sequently recovered fully. After cryptorchidism ,the TC group were treated with vitamin C (10 mg kg BW)for 8 weeks( 8) after which the animals were sacrificed by cervical dislocation. At the end of the experiment was determind of the following : each rabbit was weighed , excited testis, dissected free of surrounding tissue ,their weight determined and the volum was measured by water displacement method(9) .The testis of each rabbits were fited in 10% formalin solution, embedded in paraffin , cut in to 5µm sections and stained by haematoxylen-eosin. Then examined under light microscope .The diameters of seminiferous tubules were measured with an ocular micrometer on 20 cross section of tubules per testis.

The height of the seminiferous epithelium was taken from the base to the tubular lumen. The quantitative analysis of spermatogenesis was carried out by counting of relative number of each variety of germ cells per tubules. Apoptosis was detected in histological sections by using fuelgen reaction according to(10).The slides were observed under light microscope at 800X magnification for detection of apoptotic germ cells. Apoptotic germ cells were quantified by counting the number of fuelgen stain positive in germ cell per seminiferous tubule, the percentage of apoptotic germ cells was determined by total counting of 100 germ cells , the percentage of apoptotic seminiferous tubule was determined by counting 100 tubules for each specimen. The data were analyzed by student t- test and all results were expressed as mean  $\pm$  standard deviation. Differences between groups were considered to be significant at ( $p < 0.05$ ).

## **RESULTS**

According to the development curve recorded at the end of the 8 weeks, there are reduction in the weight gain curve after cryptorchidism in the CC group and TC group compared to the NCC group but not significantly after end of the 8 weeks (fig.1).

The weight and volume of the testis show significant decrease in the CC group compared to the NCC group ,while in the TC group was significantly elevated compared to the CC group( $p < 0.05$ ),(Tab.2).

Histological finding showed that the seminiferous tubules diameter and the height of the seminiferous epithelium was significantly depressed in the CC group compared wite NCC group ( $p < 0.05$ ), while these finding significantly elevated in the TC group compared to the CC group( $p < 0.05$ ),(Tab.3). Fig.2. showed that the mean value of the number of

germ cells present in the CC groups was significantly depressed compared to the NCC groups( $p<0.05$ ) ,while the number of germ cells was elevated significantly in the TC groups compared to the CC groups ( $p<0.05$ ) . The number of apoptotic germ cells and apoptotic tubule in the CC groups was significantly elevated compared to the NCC groups, ( $p<0.05$ )while the apoptosis was depressed significantly in the TC groups compared to the CC groups( $p<0.05$ ). ( Tab .4)

Fig .3. and fig.4. show that the percentage of apoptotic germ cells and apoptotic tubules in the CC groups was significantly higher than those in the NCC groups, while these percentages in the TC groups were significantly lower than TC group.

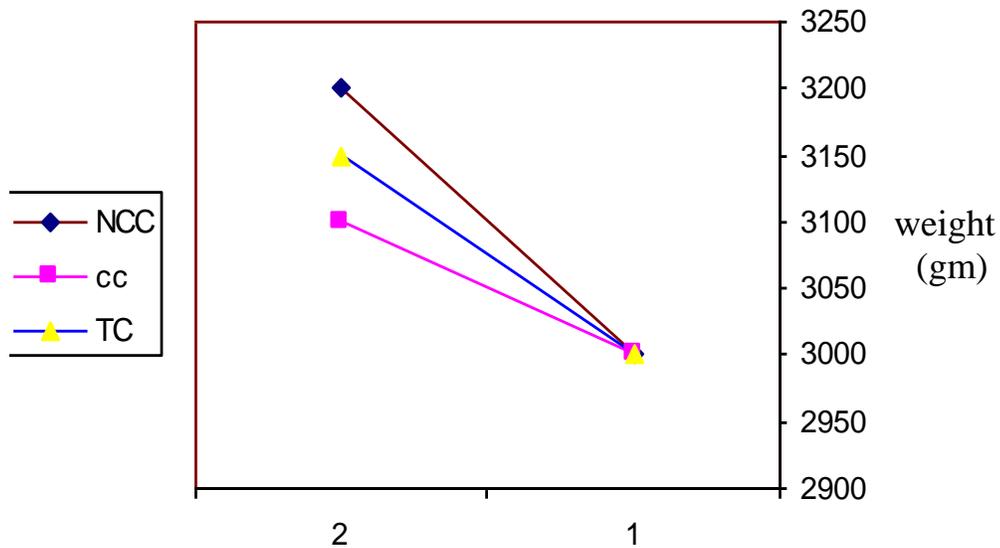


Fig.1.Body weight from initial age(1) to final age(2) In (NCC),( CC) and(TC)groups

Table .2. Volume and weight of testis of non crypt orchid (NCC), crypt orchid (CC) and treated crypt orchid (TC groups)

group	NCC	CC	TC
Testis weight(gm)	1.77±10 a	1.40±0.29b	1.73±0.10a
Testis volume(ml)	1.20±0.37a	0.91±0.005b	1.12±0.31a

Values are expressed as mean ± SD. n=10

Different vertical letters= significance differences (P< 0.05)

Table. 3.Histological finding of testis of non crypt orchid (NCC), crypt orchid (CC) and treated crypt orchid (TC groups)

parameter	NCC	CC	TC
Seminiferous tubules diameters(µm)	150±8.0 a	83±9.3 b	112±10.0 c
Seminiferous tubules epithelial high(µm)	95±2.8 a	58±6.6 b	70 ± 5.3 c

Values are expressed as mean ± SD. n=6

Different vertical letters= significance differences (P< 0.05)

Fig.2.Total Number of germ cells in  
In non crypt orchid ( NCC), crypt orchid (C) and  
treated crypt orchid (TC groups)

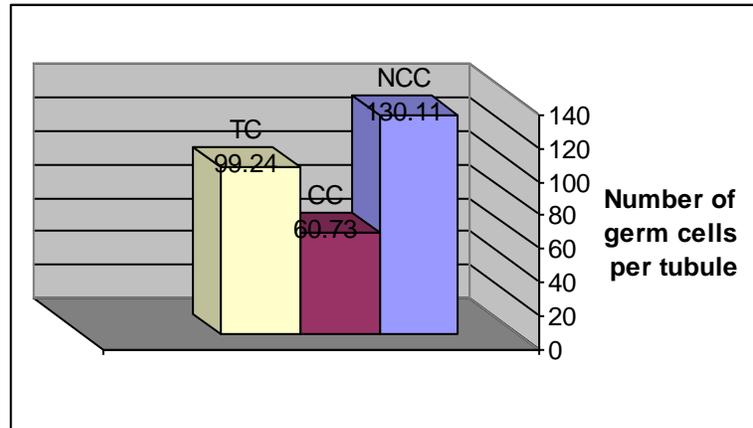


Table .4.Number of apoptotic germ cells and apoptotic tubules in  
the non crypt orchid ( NCC), crypt orchid (CC) and  
treated crypt orchid (TC groups)

Parameters	NCC	CC	TC
Number of apoptotic germ cells per tubule	11.55±2.8a	30.67±7.9b	19.20±1.6c
Number of apoptotic tubules per field	1.1±0.1a	4.60±0.1b	2.2±0.3c

Values are expressed as mean ± SD. n=10

Different vertical letters= significance differences (P< 0.0)

Fig.3. percentage of apoptotic germ cells in  
In non crypt orchid ( NCC), crypt orchid (C) and  
treated crypt orchid (TC groups)

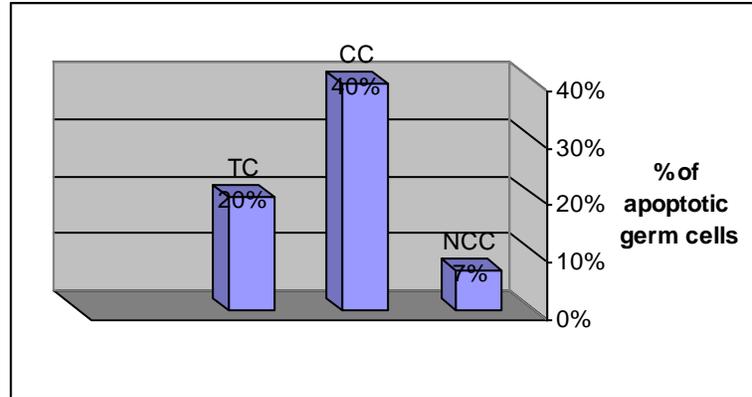
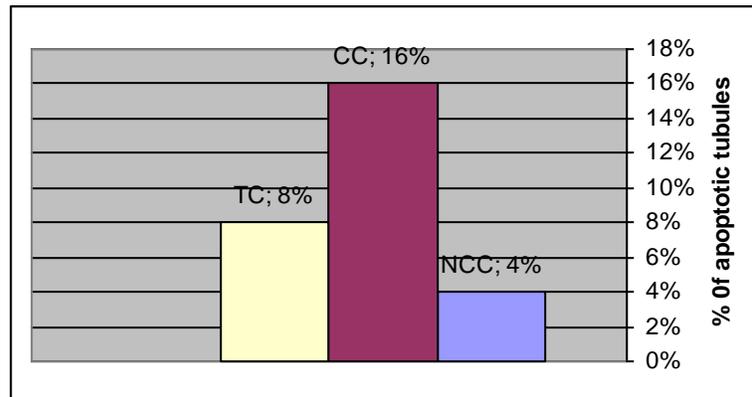
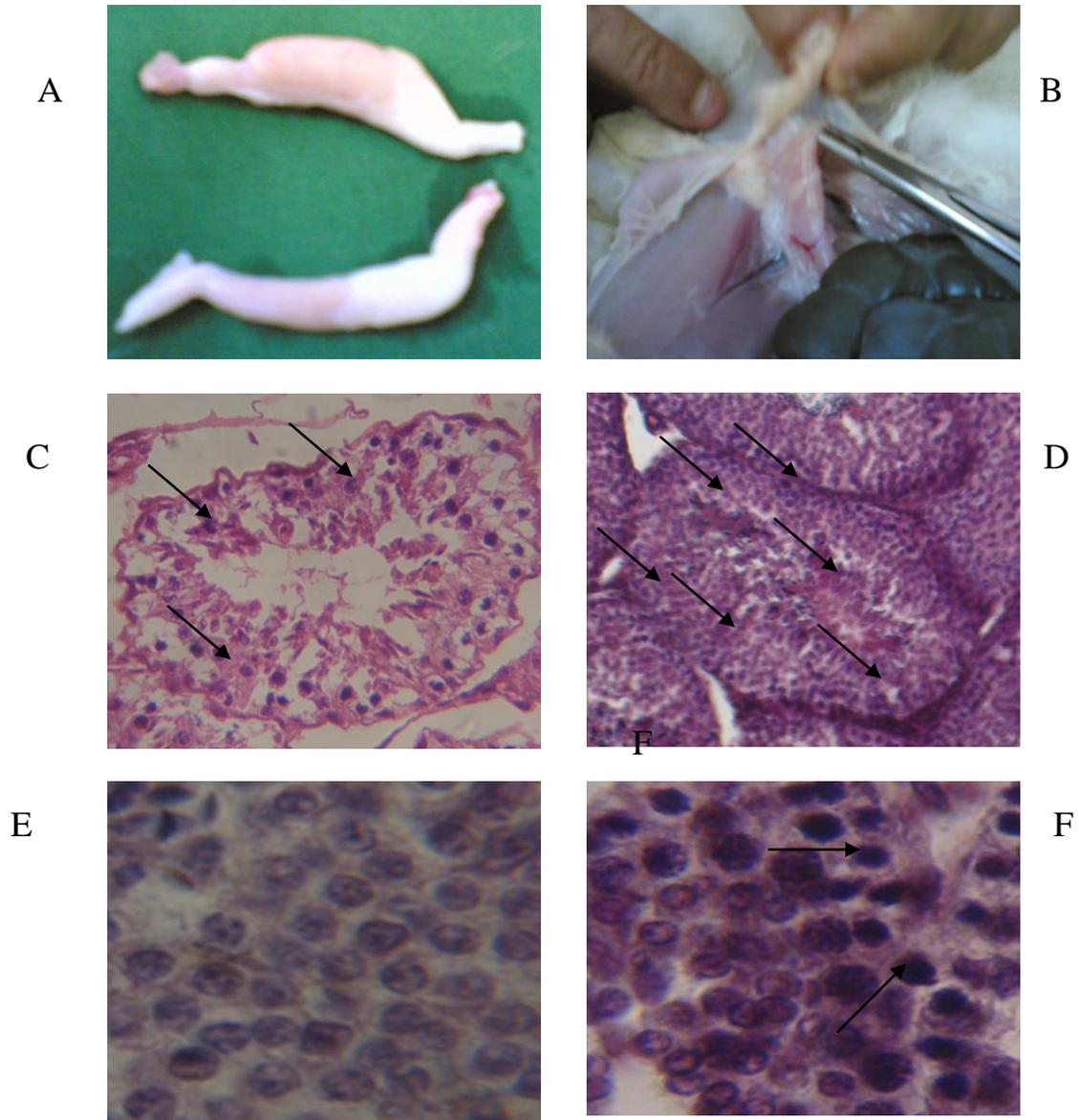


Fig.4. percentage of apoptotic tubules in  
In non crypt orchid ( NCC), crypt orchid (C) and  
treated crypt orchid (TC groups)





**Picture.1** A-control and cryptorchid testis.

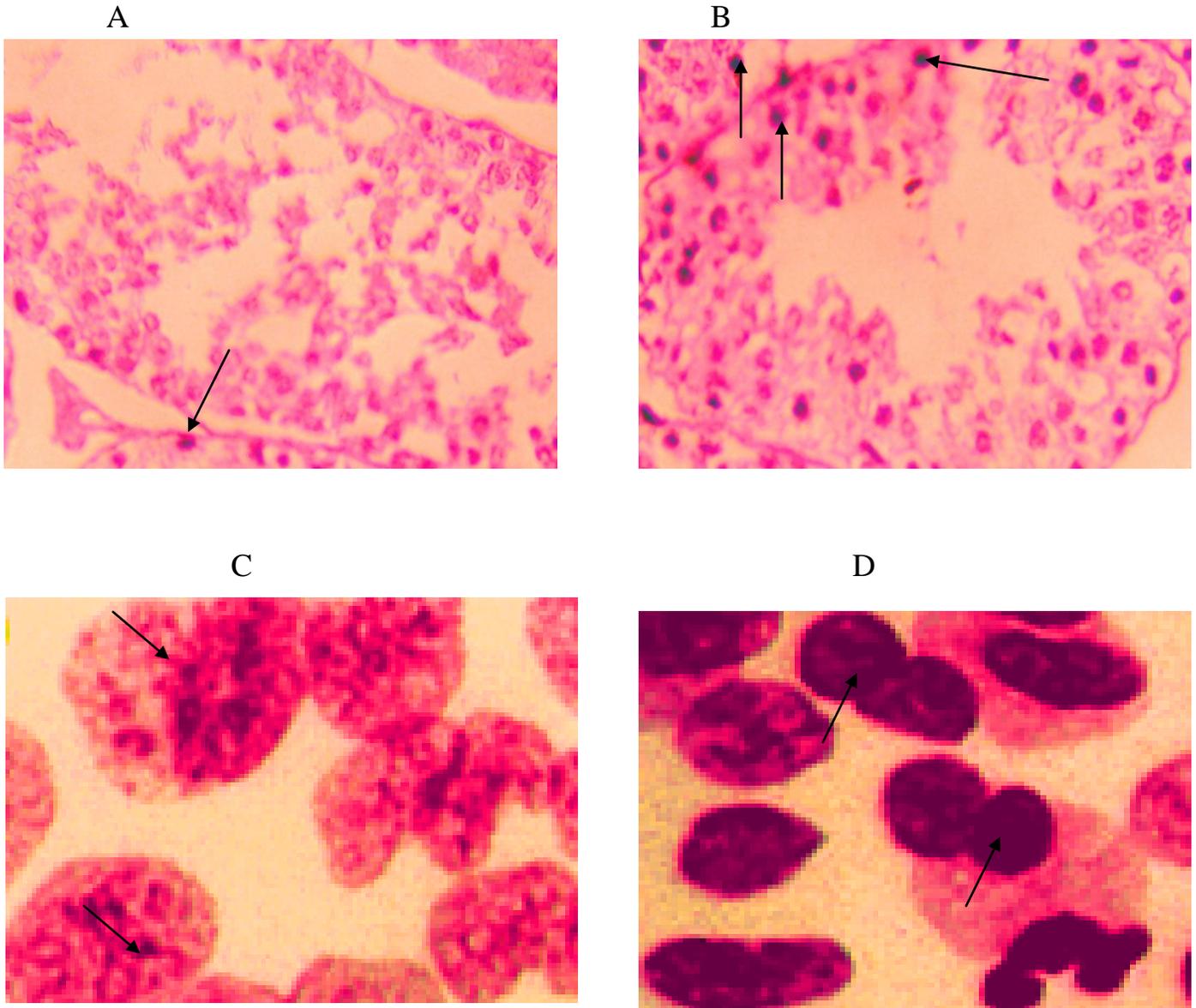
B-induction of cryptorchid testis .

C-Decrease number of germ cells in seminiferous tubule of cryptorchid testis (100X)(H.E)

D- Increase number of germ cells in seminiferous tubule of control testis(100X)(H.E)

E-Absence of apoptotic cells in seminiferous tubule of control testis(160X)(H.E)

F-Existence of apoptotic cells in seminiferous tubule of control testis(arrows)(160X)(H.E)



**Picture.2** –**A**-decrease of apoptotic cells( arrows)( fuelgen stain)(400X).  
**B**-increase of apoptotic cells( arrows)( fuelgen stain)(400X )  
**C**- Absence of apoptotic cells( arrows)( fuelge stain)(800X).  
**D**- increase of apoptotic cells( arrows)( fuelgen stain)(800X)

## DISCUSSION

The present data showed that the oxidative stress disorder of spermatogenesis and apoptosis of germ cells in the non treated cryptorchidism can be reduced by the administration of vitamin C .

The reduction in the weight gain curve after cryptorchidism can be related to decrease of intestinal absorption, provoking of reduction of essential nutrients(11).

Reduction of weight and volume for testis in the cryptorchid testis indicate that cryptorchidism causes severe impaired spermatogenesis, this is similar to other findings by(12) in human,,(12),(13) in rats and (14) in rabbits pups

The reduction in the parameters of the seminiferous tubules in the cryptorchid testis may be attributed to the reduction in the number of germ cells due to apoptosis, while,(15) attributed this reduction to the disorder in androgen .

The number of germ cells present in the cryptorchid testis of the untreated group was lower compared with the treated group. Surgical induction of cryptorchidism in experimental animals causes rapid degeneration of testicular germ cells (16). The mechanism of which has been attributed to testicular exposure to the abdominal temperature(17). The high temperature stress which is associated with experimental cryptorchidism is associated with oxidative stress in the testis and a reduction in reactive oxygen species(ROS) which are highly reactive because of an unpaired electron(6). In a normal state there is equilibrium between the generation of ROS required for normal sperm function. Excessive production of ROS results in destruction of the antioxidant capacity of sperm causing oxidative stress which damages spermatozoa membranes(18). The damage to the germ cells via a mechanism that is associated with elevated  $H_2O_2$  generation and could be ameliorated by addition of catalase with axanthin and xanthine oxidase(19)

,(20) reported that a low dose of testosterone in the cryptorchid testis suppresses spermatogenesis in rat and monkey. It suggests that down-regulation of phosphodiesterase type 4 enzyme(PDE4) expression in the cryptorchid testis may play an important role in the degeneration of germ cells(21,22)

The increase in the mean value of the number of germ cells present in the TC group indicates that vitamin C was able to ameliorate the sequence of cryptorchidism and reduce

testicular germ cells apoptosis which has been show in this study. This results are similar to the finding that the administration of ascorbic acid improve sperm quality in smoker and in fertile men(23). Several studies have show that vitamin C counteract the testicular oxidative stress induced by exposure to pro-oxidants(John and Shaun,2005),also it was reported that deficiency of vitamin C lead to state of oxidative stress in the testis that disrupt both spermatogenesis and the production of testosterone.( 6) show that degeneration of spermatocytes occurred frequently in vitamin c deficient mice. it is expected that antioxidant therapy will act as a protective defense against oxidative stress(24).

A number of no enzyme factors also function as antioxidants in the testis , among these vitamin E, reversal role, melatonin and Trino IB (7).

Oxidative stress in the testis is one of the major factor that induce germ cells apoptosis. The antioxidants act to protect of germ cells against oxidative DNA damage(25) and play important role in spermatogenesis .in fuelgen reaction, Schiff's reagent will bind to the exposed aldehyde at the 3 end of the deoxyribose sugar staining the apoptotic germ cells as brick red in color(10).

### تأثير فيتامين س على التموت المبرمج للخلايا الجرثومية للخصية في الارانب

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#### الخلاصة

بينت الدراسة تأثير فيتامين س على التموت المبرمج للخلايا الجرثومية الخصوية في ذكور الارانب المعرضة لحالات احتباس الخصية التجريبي.أحدث التجريع الفموي لفيتامين س ( 10 ملغم/كغم من وزن الجسم) لمدة لمدة ثمانية اسابيع ارتفاع معنوي في القياسات الخصوية في المجموعة المعالجة وايضا ارتفاع معنوي لعدد الخلايا الجرثومية مقارنة مع المجموعة الغير معالجة .كذلك ادى الى هبوط الخلايا الجرثومية المتماوتة والأنابيب المنوية المتماوتة في المجموعة

المعالجة مقارنة مع المجموعة غير المعالجة. ان فيتامين س خفف الإجهاد التأكسدي الناتج عن احتباس الخصية التجريبي.

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