

Effect of Vitamin C on Epididymal Sperm Quality in the Rat Experimentally Induced Unilateral Cryptorchidism

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Abstract

Objective- Release of free radicals and oxidants following increased temperature of testis in cryptorchidism, play an important role in the producing testicular damage. The present study investigated the role of vitamin C as an antioxidant in protecting the testis against damage in experimental unilateral cryptorchidism.

Design- Experimental study

Animals- Forty five male adult Wistar–Albino rats

Procedures- Animals were divided into three groups. The control group had intact rats, the cryptorchid group had unilateral cryptorchid rats in left testis and the treatment group had unilateral cryptorchid rats in left testis that it received vitamin C at a dose of 50 mg/kg body weight intraperitoneal, every other day, during experimental period. Epididymis samples were obtained from both testes of five cases of 15 animals of each group at 15, 30 and 60 days after induction of cryptorchidism and testicular weight/body weight ratio and epididymal sperm characteristics (sperm concentration, sperm motility and sperm viability) were evaluated.

Results- Testicular weight/body weight ratio and sperm quality of the cryptorchid (left) testes in cryptorchid group significantly decreased compared to the control group ($p < 0.05$), and treatment with vitamin C not significantly improved these parameters of these testes compared to the cryptorchid group ($p > 0.05$). In addition, the left testes on unilateral cryptorchid rats had noticeable adverse effects on the sperm quality parameters

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of scrotal (right) testes ($p < 0.05$), and treatment with vitamin C after 60 days, significantly improved all sperm quality parameters of these testes compared to the cryptorchid group ($p < 0.05$).

Conclusion and clinical relevance- It can be concluded that although treatment with vitamin C has few positive effect on sperm quality of cryptorchid testes, but it can significantly improve these parameters in scrotal testes on unilateral cryptorchid rats. Therefore it may be useful for treatment of cryptorchidism outcomes in male animals.

Keywords: Cryptorchidism, Vitamin C, Sperm quality, Rat.

Introduction

Cryptorchidism occurs when the normal process of testicular descent is perturbed, such that one or both testes fail to complete their descent into the scrotum.^{1,2,3,4} It's the most frequent abnormality of male sexual differentiation.^{5,6,7,8} Cryptorchidism occur in all domestic species, but is seen most commonly in the stallions, boars and some breeds of dogs, less commonly in bucks and rams, uncommonly in bulls and rarely in tom cats.^{1,9,10}

The lower levels of the scrotal temperature are believed to maintain optimal environment for testicular function, and it is known that surgical induction of cryptorchidism in experimental animals causes rapid degeneration of testicular germ cells and disruption of spermatogenesis because of the higher temperature in the abdominal cavity.^{1,2,11,12} Cryptorchidism, if bilateral, results in sterility.^{1,8,9,10} Unilateral cryptorchidism is more common and usually results near normal fertility because of normal sperm production from the testis located in the scrotum.¹⁰ The effect of unilateral cryptorchidism on fertility has been more controversial as the undescended testis is proposed to have a compounding effect on the contralateral descended testis.^{2,3,13}

Studies have shown that increasing testicular temperature above normal levels results in altered spermatogenesis due to effect of heat which brings about oxidative stress on the seminiferous tubules.^{4,12,14} Oxidative stress in biological systems originates as the result of an imbalance between the generation of oxidizing species and cellular antioxidant defences.^{15,16,17} The generation of oxidative free radicals occurs constantly during normal cell metabolism in all living cells, and their effects are counterbalanced by the endogenous antioxidant system.^{18,19} When generation of this oxidizing species exceeds the defense mechanisms capacity to control, oxidative stress is generated and contributes to reversible or irreversible cell injury.^{4,12,16,17} It can oxidize cell membrane lipids, proteins and DNA, leading to cellular dysfunction and sometimes cell death.^{13,20} It had been shown that the elimination of ROS (Reactive Oxygen Species) can be as an important factor in treating side-effects of cryptorchidism.^{5,12,14,21}

Antioxidants, which enhances the endogenous antioxidant defense systems within cells^{14,18}, can protect against the damaging effect of ROS on testes and may be of clinical value in assisted conception procedures.²¹ In the body of animals, vitamin C is one of the most important and most prevalent water-soluble antioxidants.^{15,22,23,24,25} As a water-soluble antioxidant, it exists inside the cells and in the extracellular matrix and scavenges free radicals that may damage to existing structures in these locations.²⁶ Vitamin C also indirectly protects the cell membranes and lipid-based structures along with Vitamin E.^{22,24} It is an electron donor and therefore a reducing agent.^{23,25}

The aim of the current study was to evaluate the sperm quality in cryptorchid and scorotal testes after administration of vitamin C in the rat experimentally induced unilateral cryptorchidism.

Materials and Methods

Animals

All animal experiments in this study were approved by the Animal Ethics Committee at the Veterinary Faculty of Kerman. Forty five adult male Wistar–Albino rats (250–300 g) were obtained from Neuroscience Research Center of Kerman, Iran. The rats were fed with standard commercial laboratory chow [(pellet form), Javaneh Khorasan Co., Mashhad, Iran] and water ad libitum and housed under standard laboratory conditions (12 hours light, 12 hours dark and 22 ± 2 °C) during the experimental period.

Experimental protocol

Animals were weighed and divided randomly into three groups each containing fifteen rats. Group A served as the "Control" and the rats were neither rendered cryptorchid nor treated with Vitamin C which was for determination of the basal values for all parameters. Groups B (Cryptorchid) and C (Treatment) served as the experimental groups which the rats rendered unilaterally cryptorchidism in the left testes. Group C in addition had 50 mg/kg body weight of vitamin C (Daroupakhsh Co., Tehran, Iran) injected intraperitoneally after the surgically induced cryptorchidism every other day for 60 days. The animals in groups A and B were received normal saline intraperitoneally exactly at the same volume and injection interval as group C. The control, cryptorchid and treatment animals were euthanized with sodium thiopental (Sondos Co, Austria) on days 15, 30 and 60 after operation, five rats at each evaluated time points, then animals were weighed and both testes and epididymises were removed for testicular weight/body weight ratio and caudal epididymal sperm characteristics evaluations.

Surgical technique

To induce cryptorchidism, the rats were anaesthetized with intraperitoneal injection of 10 mg/kg body weight xylazine hydrochloride (Alfasan Co., Netherlands) and 100 mg/kg body weight ketamine hydrochloride (Alfasan Co., Netherlands). Then, the skin of the scrotal region was shaved and prepared by povidon-iodine solution. Left inguinoscrotal region was incised and the ligament of tail of epididymis was separated. The freed testis was pushed back into the abdominal cavity through the internal inguinal ring. After pushing the testis, the external inguinal ring was closed by 4/0 nonabsorbable suture material (Nylon; Supa Co., Iran).²⁷

Testicular weight/body weight ratio

To calculate the ratio of testicular weight/body weight, animals were weighed after euthanasia and both testes were removed. Adipose tissues around the testes were separated and then testes were weighted with sensitive weighing scale (Sartorius Co., Germany). This value was expressed as the percent of testicular weight/body weight ratio.

Sperm quality analysis

Sperm samples were obtained from five cases of 15 animals of each group during experimental period at 15, 30 and 60 days. Samples of mature spermatozoa were collected from the caudal region of epididymis by mincing it finely in PBS at 37°C. Sperm quality was determined by three parameters: sperm concentration, motility and viability.

Sperm concentration was analyzed using the haemocytometer method (World Health Organization, 1999). Sperm suspensions from the caudal epididymis were diluted 1:100 with PBS and transferred into microcentrifuge tubes. The diluted samples were put into the counting chamber, and the numbers of spermatozoa were counted using a haemocytometer with improved double Neubauer ruling under a light microscope. The sperm concentration was expressed as $\times 10^6 \text{ ml}^{-1}$.

Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa under a light microscope equipped with a hot plate and expressed as the percent motility (World Health Organization, 1999).

Sperm viability was performed by the eosin-nigrosin staining (World Health Organization, 1999). One drop of sperm suspensions was mixed with two drops of 1% eosin Y. After 30 seconds, three drops of 10% nigrosin were added and mixed well. A smear was made by placing a drop of mixture on a clean glass slide and allowed to air dry. The prepared slide was examined. Pink-stained dead spermatozoa and unstained live spermatozoa were counted under the light microscope. The viability of spermatozoa was expressed as the percent of viable spermatozoa.

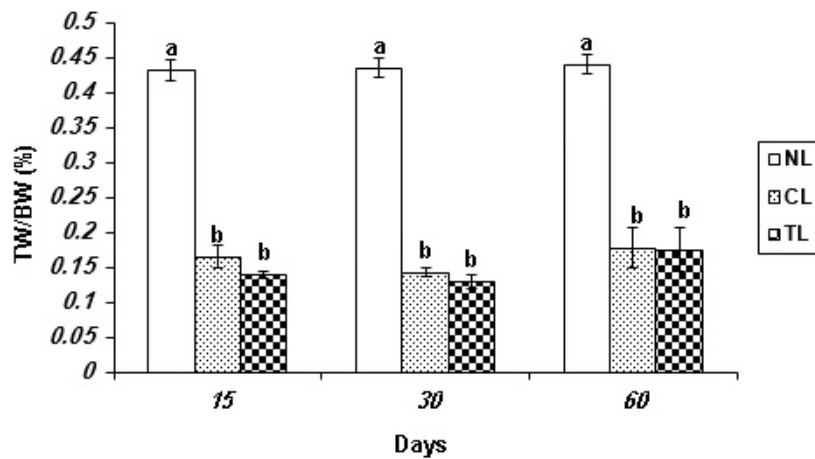
Statistical analysis

Values were expressed as mean \pm SEM (Standard Error of Mean). Statistical evaluation of significant difference between means was performed with one-way analysis of variance (ANOVA) followed by the Tukey's multiple range test. The significance level considered was $P < 0.05$.

Results

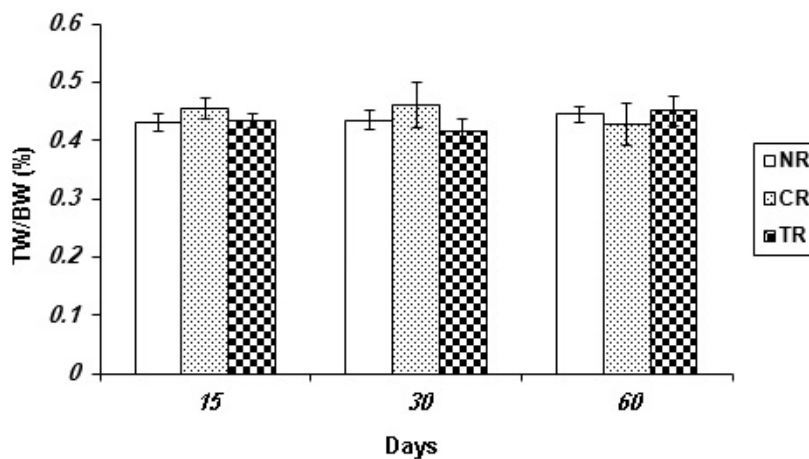
Testicular weight/body weight ratio

Although induction of cryptorchidism in the left testes significantly decreased the percent of testicular weight/body weight ratio compared to the control group ($P < 0.05$), but not affected contralateral (right) testes at each evaluated time points ($P > 0.05$) (Graph 1 & 2). Administration of vitamin C also not significantly improved this ratio in cryptorchid and scrotal testes compared to the cryptorchid group ($p > 0.05$).



Graph 1: Mean ± SEM percentage of testicular weight/body weight ratio in left (cryptorchid) testes following experimental unilateral cryptorchidism in rats. Control group (NL), Cryptorchid group (CL), Treatment group (TL)

^{a,b} Different superscript alphabets show significant difference for each evaluated time points ($P < 0.05$).

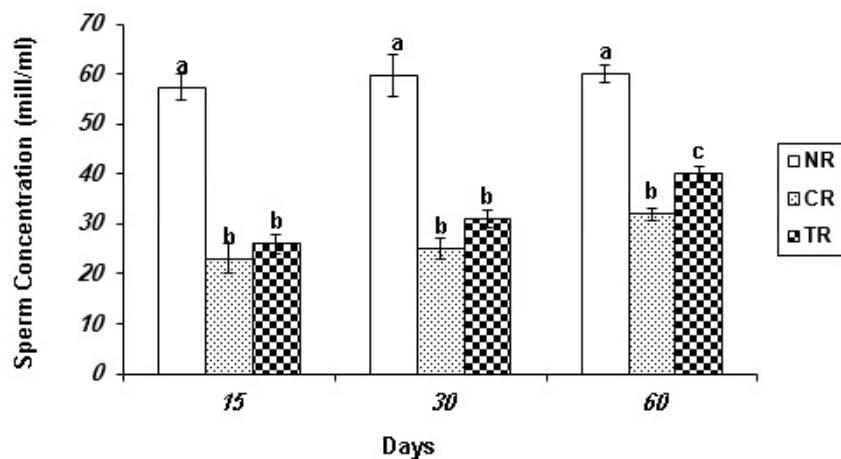


Graph 2: Mean ± SEM percentage of testicular weight/body weight ratio in right (scrotal) testes following experimental unilateral cryptorchidism in rats. Control group (NR), Cryptorchid Group (CR), Treatment group (TR). There are no significant difference between the groups ($P > 0.05$).

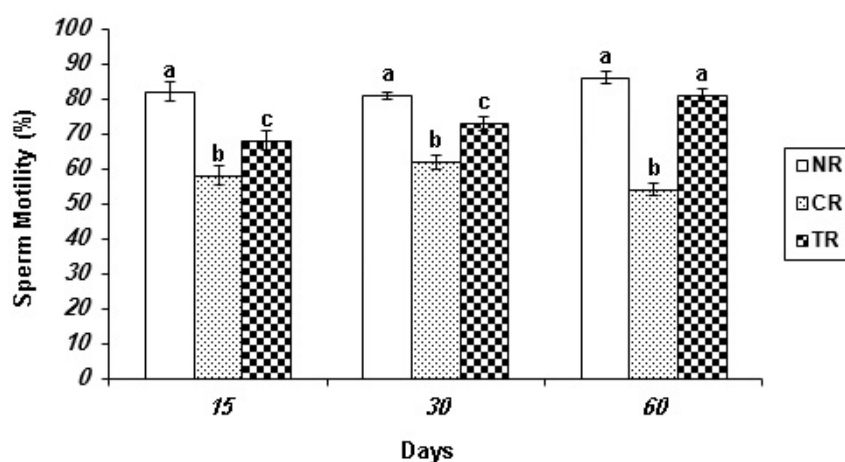
Sperm quality analysis

In the left testes experimental cryptorchidism caused a severe and significant damage to the spermatogenesis process so that the sperm concentration was reached to less than one million per ml and there are no motile and viable sperms at each evaluated time points. Also administration of vitamin C could not improve above mentioned parameters.

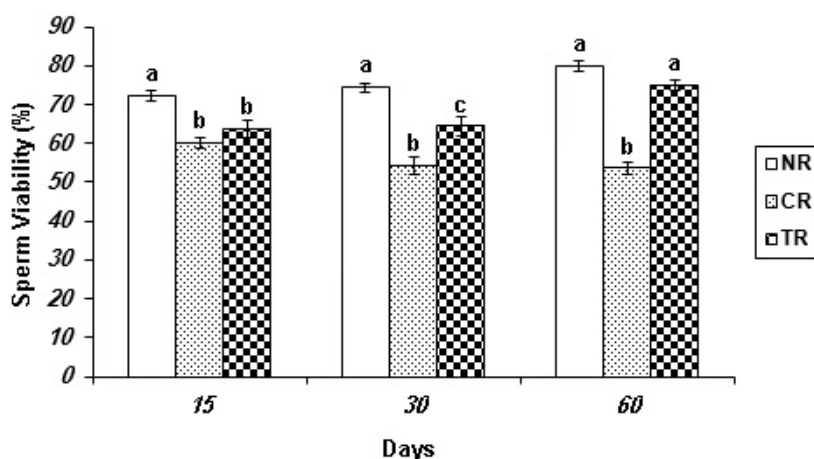
In the right testes all values of sperm concentration, sperm motility and sperm viability significantly decreased in the cryptorchid group compared to the control group at each evaluated time points ($P < 0.05$) (Graph 3, 4 & 5). Administration of vitamin C significantly improved deleterious effects of cryptorchidism at 60 days in sperm concentration, 30 and 60 days in sperm viability and each evaluated time points in sperm motility after operation compared to the cryptorchid group ($P < 0.05$). Also the values of sperm motility and sperm viability reached to the control group after 60 days of Vitamin C therapy ($P > 0.05$).



Graph 3: Mean \pm SEM sperm concentration (mill/ml) in right (scrotal) testes following experimental unilateral cryptorchidism in rats. Control group (NR), Cryptorchid group (CR), Treatment group (TR).
^{a,b,c} Different superscript alphabets show significant difference for each evaluated time points ($P < 0.05$).



Graph 4: Mean \pm SEM percentage of sperm motility in right (scrotal) testes following experimental unilateral cryptorchidism in rats. Control group (NR), Cryptorchid Group (CR), Treatment group (TR).
^{a,b,c} Different superscript alphabets show significant difference for each evaluated time points ($P < 0.05$).



Graph 5: Mean \pm SEM percentage of sperm motility in right (scrotal) testes following experimental unilateral cryptorchidism in rats. Control group (NR), Cryptorchid Group (CR), Treatment group (TR).
^{a,b,c} Different superscript alphabets show significant difference for each evaluated time points ($P < 0.05$).

Discussion

Surgical induction of cryptorchidism has been frequently used as a model for the study of heat stress on testicular tissue.^{3,4,5,18} In present study, we used from the Dundar *et al.* surgical method for induction of cryptorchidism in rats.²⁷ It has been generally accepted that a relatively low temperature is preferable for testicular function of most mammalian species and increasing temperature above normal levels, following the cryptorchidism, results to disruption of spermatogenesis.^{1,2,11,12,28} Many studies have expressed that deleterious histological changes occur on the testis after induction of cryptorchidism.^{3,4,29,30,31,32} Our study with data showed severe changes and significant reduction in the testicular weight/body weight ratio and amounts of all sperm characteristics (sperm concentration, sperm motility and sperm viability) in cryptorchid testes, therefore corroborates these findings. It is because of that the cryptorchidism is associated to a decrease in antioxidant enzyme activity^{33,34}, or an increase in the production of ROS, like superoxide anion, hydroxyl radical, nitric oxide and hydrogen peroxide in response to heat stress^{12,35,36,37}, which stimulate lipoperoxidation and activating the apoptosis.^{14,16,18,38,39}

Treatments with antioxidants can increase the endogenous antioxidant defense system of the cells, inhibiting via that lead to ROS production or working to trap free radicals and impede lipoperoxidation.^{21,38} Numerous antioxidants have been used to lessen the damage caused by free radicals in male infertility and cryptorchidism, such as vitamin E, xanthine oxidase, nitric oxide synthase inhibitor, glutathione, coenzyme Q10 and zinc.^{4,12,14,21,40,41} but there have been no reports about the use of vitamin C in this pathology. The beneficial influences of vitamin C can be attributed to the fact that it's a very efficient antioxidant, and a scavenger of oxygen free radicals^{21,22,23,25,26}, and it's important in maintaining the physiological integrity of testis, epididymis and accessory glands.²¹ For these reasons, the present study was planned to evaluate the role of vitamin C as a protective agent against testicular oxidative stress on ipsilateral and contralateral testicular function. Saalu *et al.* (2007) and Viguera *et al.* (2011) have expressed that administration of vitamin E in cryptorchidism conserving the spermatogenesis and increasing fertility^{4,14}. Whereas Komagai *et al.* (2002) and DeFoor *et al.* (2004) have suggested that inhibition of ROS with xanthine oxidase or nitric oxide synthase inhibitor treatments in cryptorchidism partially rescues the spermatogenic damage but the animals are still infertile.^{12,40} The results of this study demonstrated that although administration of vitamin C after 60 days don't improved sperm characteristics in cryptorchid testes and the spermatogenesis process still will be arrested which confirm Komagai and DeFoor reports. It may be due to that these treatments raise the possibility to prevent at least some germ cell death in cryptorchid testes by interfering with the apoptotic machi.⁵ Also many researchers have shown that primary spermatocytes and early spermatids are the most heat sensitive cell types in testis^{5,39,42,43}, these can be another explanation that why were in our results the values of sperm concentration very low and there were not motile and viable sperm in cryptorchid testes.

The detrimental effects of unilateral cryptorchidism on the contralateral testes have been described by many authors in relation to subfertility.^{2,3,8,29,31,32,46} In our study all sperm characteristics have showed moderate decrease in their values. Although the effect of unilateral cryptorchidism on ipsilateral testicular function may be attributable to the increase in ipsilateral testicular temperature, there is much controversy concerning the mechanisms that mediate the detrimental effect of unilateral cryptorchidism on contralateral testicular function.^{8,29,31,32} It has demonstrated an increase in contralateral testicular blood flow and temperature in animals with unilateral cryptorchidism.^{2,3,46} Moreover, the produced free

radicals in ipsilateral testis are the most important source which may receive to the contralateral testis by blood flow.¹⁷ These can be explanations for moderate changes of sperm characteristics which were observed in our experiment. Our results showed that treatment with vitamin C after 60 days has a positive effect on all sperm characteristics following induction of cryptorchidism in contralateral testes.

Conclusion

According to the results of present study, it could be concluded that experimental unilateral cryptorchidism induced adverse effect on sperm quality of both cryptorchid and scrotal testes. Administration of vitamin C improved sperm quality of scrotal testes significantly but its beneficial effects on sperm characteristics of cryptorchid testes were limited.

Acknowledgement

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بررسی اثر ویتامین ث بر روی کیفیت اسپرم اپیدیدیمی متعاقب القاء نهان بیضگی یک طرفه تجربی در موش صحرائی

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هدف - آزاد شدن رادیکال‌های آزاد و عوامل اکسیدان متعاقب افزایش درجه حرارت بیضه متعاقب نهان بیضگی نقش مهمی را در آسیب به بافت بیضه ایفاء می‌کند. مطالعه حاضر نقش ویتامین ث به عنوان یک عامل آنتی‌اکسیدان در حفاظت از بافت بیضه در نهان بیضگی یک طرفه تجربی را مورد بررسی قرار می‌دهد.

طرح مطالعه - مطالعه تجربی

حیوانات - ۴۵ سر موش صحرائی سفید نژاد ویستار-آلبینو

روش کار - حیوانات به سه گروه مساوی تقسیم شدند. گروه شاهد: هیچ عمل جراحی بر روی حیوانات انجام نشد، گروه کریپتورکید: حیوانات تحت عمل جراحی نهان بیضگی یک طرفه بروی بیضه چپ قرار گرفتند و گروه درمان: مشابه گروه قبل جراحی شده و ۵۰ میلی‌گرم به ازای هر کیلوگرم وزن بدن ویتامین ث یک روز در میان به صورت تزریق داخل صفاقی در طول دوره مطالعه دریافت نمودند. نمونه اپیدیدیمی از بیضه ۵ سر از ۱۵ سر موش صحرائی هر گروه در روزهای ۱۵، ۳۰ و ۶۰ بعد از جراحی تهیه شد. در این مطالعه نسبت وزن بدن به وزن بیضه و خصوصیات اسپرم (غلظت اسپرم، تحرک اسپرم و زنده ماندن اسپرم) در بین گروه‌ها مورد ارزیابی قرار گرفت.

نتایج - نسبت وزن بیضه به وزن بدن و کیفیت اسپرم در بیضه‌های سمت چپ (نهان بیضه) در گروه کریپتورکید به طور معنی‌داری نسبت به گروه شاهد کاهش یافت ($P < 0.05$) و درمان با ویتامین ث تاثیر معنی‌داری بر بهبودی این بیضه‌ها نداشته است ($P > 0.05$). علاوه بر این نهان بیضگی در بیضه سمت چپ اثرات مخربی بر روی کیفیت اسپرم در بیضه‌های سمت راست (داخل اسکروتوم) داشت ($P < 0.05$). درمان با ویتامین ث بعد از ۶۰ روز به طور معنی‌داری اثر بهبودی بر کیفیت اسپرم بیضه‌های سمت راست (داخل اسکروتوم) نسبت به بیضه‌های مشابه در گروه کریپتورکید داشته است ($P < 0.05$).

نتیجه گیری و کاربرد بالینی - بر اساس نتایج این مطالعه چنین می‌توان نتیجه‌گیری نمود که اگرچه ویتامین ث اثر درمانی ناچیزی بر کیفیت اسپرم بیضه‌های مبتلا به نهان بیضگی دارد اما اثر بهبودی معنی‌داری را بر روی کیفیت اسپرم بیضه‌های طرف مقابل (داخل اسکروتوم) در موش‌های مبتلا به نهان بیضگی یک طرفه نشان می‌دهد. بنابراین تجویز ویتامین ث ممکن است برای درمان بیماران مبتلا به نهان بیضگی یک طرفه مفید باشد.

کلید واژگان - نهان بیضگی، ویتامین ث، کیفیت اسپرم، موش صحرائی.