# Effects of L-Arginine, Vitamin E and Their Combinations on Sperms Morphology in Albino Male Mice

Raza H. Husein, Mahmood O. Ahmed and Sulaf M. Muhammed
Biology Department, College of Science, Sulaimani University.
E-mail : razahassan72@yahoo.com.

**Abstract**

This study designed to evaluate the effected of L-arginine, vitamin E and their combinations on sperms morphology (sperm quality). Twenty (20) male albino mice was included in this study and dividing into four groups (Control group, Vitamin E treated group, L-arginine treated group, and Vitamin E+ L-arginine treated Group). The animals were kept under suitable conditions of temperature (24±2 ˚C) and light program (12-14 hours/ day).

Results revealed that mice treated with vitamin E + L-arginine (0.80 ± 0.141) showed a significant ($p \leq 0.05$) decrease in numbers of abnormal sperm without head, treatment mice with vitamin E + L-arginine (1.68 ± 0.10) gave a significant decreased in numbers of abnormal sperms without tail, mice treated with vitamin E (1.00 ± 0.38) and (L-arginine + vitamin E) (0.52 ± 0.14) significantly decreased sperms without hook, furthermore mice treated with vitamin E (11.04+1.63) significantly decreased sperms defective head when compared with control groups. the period of experiments were 30 days. The percentages of abnormal sperm morphology were calculated by counting 200 spermatozoa**.**

Keywords: sperm abnormality, L-arginine, vitamin E, L-arginine +vitamin, albino male mice.

## Introduction

L-arginine plays a key role in modulating host defences and cellular immunity and L-arginine is considered a semi-essential amino acid because even though the body normally makes enough of it, supplementation is sometimes needed. It also actively participates in sperm formation [1]. A deficiency in L-arginine causes derangement of sperm metabolism leading to decrease in motility, loss of spermatogenesis and increase morphology abnormality [9]. Administration of L-arginine to oligospermic and asthenospermic patients results in an improvement in both the sperm count and motility without any side effects [2]. Furthermore L-arginine plays an important role in stimulating sperm motility in humans, rabbits, and goats under *in vitro* conditions [17]. In earlier publications, have shown that L-arginine enhances the rate of glycolysis, resulting in higher rates of ATP and lactate generation in spermatozoa [16].

Environmental factors, such as pesticides, exogenous estrogens, and heavy metals may negatively impact spermatogenesis. A number of nutritional therapies have been shown to improve sperm counts and sperm motility, including carnitine, arginine, zinc, selenium, and vitamin B-12. Numerous antioxidants have also proven beneficial in treating male infertility, such as vitamin C, vitamin E, glutathione, and coenzyme Q10. As well as specific botanical medicines, have been documented in several studies as having a positive effect on sperm parameters [20]. Arginine supplementation significantly improved sperm motility and abnormality without any side effects (Scibona *et al*. 1994).

Vitamin E (VE) is a well-known antioxidant and an effective primary defense against lipid peroxidation of cell membrane [15]. Vitamin E comprises 8 natural fat-soluble compounds, including 4 tocopherols and 4 tocotrienols. Among them, a-tocopherol is the most prevalent and the most active. Due to its effective antioxidant property and free radical scavenging capability, administration of a-tocopherol has been proposed as a potential radio-protectant, and lipid peroxidation in the testis of mice [20].

## Materials and Methods

### Animals

Albino male mice of 8 weeks age were housed in plastic cages measuring about (29×15×12) cm, with five mice per cage. Floors of cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study.

Animals were kept in the animal house of Sulaimani university in an air conditioned room with an optimum temperature of 24±2 ˚C and exposed to about 12-14 hrs/day light program. These conditions represent the optimum environmental conditions which are required by mice. During the period of the experiment abnormal and sick animals were excluded. Water and food were locally prepared and consisted of available constituents which fulfill the mice dietary requirements.

### Experimental Design

This study included twenty mature albino male mice devided into four groups as follows:

1-**Control group**: this group was orally administrated with tap water during the 30 days period of the experiment.
2-**Vitamin E treated Group**: this group was orally administrated with 500 IU of vitamin E during the six weeks period of the experiment.
3-**L-arginine treated Group**: this group was orally administrated with 0.1mg/100ml water of L-arginine during the 30 days period of the experiment.
4-**Vitamin E+ L-arginine treated Group**: This group was orally administrated with 500 IU of vitamin E and 0.1mg/100ml water of L-arginine during the 30 days period of the experiment.

At the end of the period of treatment, 5 males per group were sacrificed by cervical dislocation for the evaluation of sperm functions, using both right and left cauda (Epididymis tail) of each male, and for assessment of reproductive hormonal analysis (LH, and Testosterone). Each of collected (testes and epididymes) were kept in formal saline 10% then after 16-18 hrs they were replaced in 70% alcohol for preservation till the time of analysis. Using a small pipette, a drop (50 µl) of spermatozoal suspension was placed on a microscopical glass slide and then covered with a coverslip, then left for one minute in incubator to stand before microscopic examination. Concentration of spermatozoa (sperm/ml) was calculated from the mean number of spermatozoa of 10 randomly selected microscopical fields under high power magnification (400X). The number obtained was multiplied by a factor of one million), this estimation was repeated twice for each sample and average was taken.

Total spermatozoa concentration = No. of spermatozoa×multiplication factor (1 million). All images were obtained by using advanced digital camera.

### Abnormal sperm morphology

Using the same slide which was used for estimation of sperm concentration, the sperm morphological abnormalities were observed in each part of spermatozoa (head, tail and midpiece), and then a picture was taken showing different sperm morphological abnormalities.

### Statistical analysis

Analysis of data was performed by using SPSS (Version 15). Results are expressed as mean+standard error (M±S.E.) Statistical differences were determined by Duncan test for multiple comparisons after ANOVA. $p \leq 0.05$ was considered statistically significant.

## Results

Sperm morphology showed hook-shape heads; sperm morphology is routinely evaluated as part of a standard seminal analysis. Morphology refers to the size and shape of the sperm. The results of a sperm morphology exam indicated the percentage of sperm that appear normal when semen is viewed under a microscope.

### 1- Sperm without head

The treated male albino mice with L-arginine (7.40 ± 1.19) and vitamin E (7.24 ± 0.97) showed no significant differences ($p \leq 0.05$) in the abnormal sperm without head in comparison to the control group (8.28 ± 1.53) while treatment with (vitamin E + L-arginine) (0.80 ± 1.41) significantly ($p \leq 0.05$) decreased the numbers of abnormal sperms

without head when compared with control group for the same period (Table (1), Fig.(1)).

**2- Sperm without tail**

Results showed that mice treated with L-arginine (6.88±1.44) and vitamin E (8.68 ± 1.30) statistically not decreased (P≤0.05) in sperms without tail when compared with the control groups (7.36 ± 1.20). However, treatment of mice with (vitamin E + L-arginine) (1.68 ± 0.10) significantly decreased the numbers of abnormal sperm without tail when compared with control group (Table (1), Fig.(1)).

**3- Sperm without hook**

The obtained results showed that there were no significant differences in number of sperms without hook between the control group (4.68 ± 0.21) and treated mice with L-arginine (3.14 ± 0.39), whereas, mice treated with, vitamin E (1.00 ± 0.38) and (L-arginine+vitamin E) (0.52 ± 0.14) gave a significant decrease in number of sperm without hook respectively in comparison to the control group (Table (1), Fig.(1)).

**4- Sperm with defective head**

Results in Table (1) indicates that there was no significant difference in number of sperms with defective head between the control group (5.04± 0.40) and mice treated with L-arginine (7.72± 0.94) whereas, mice treated with, vitamin E exhibited a significant increase in numbers of sperm with defective head (11.04±1.63) in comparison with the control group. Moreover mice treated with (L-arginine+vitaminE) showed a significant decrease in numbers of sperm with defective head (0.240± 0.11) in comparison to the control (Table (1), Fig.(1)).

**Effects of L-arginine, vitamin *E* and both interactions on LH hormone in mice.**

**LH hormone**

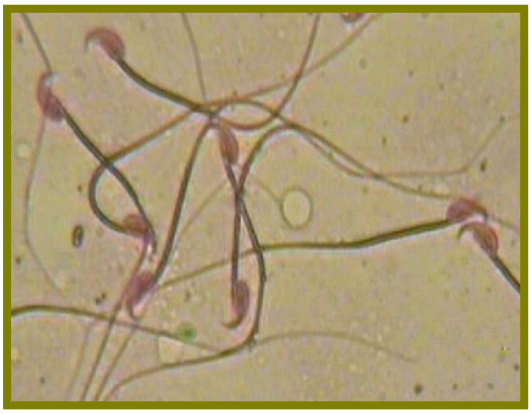
Results showed that serum levels LH hormone in mice treated with L- arginine and vitamin E (0.84±0.020 and 0.87±0.031) were significantly lower when compared with the control group (1.69±0.20). However, mice treated with, (L-arginine+vitaminE) (1.51±0.19), were significantly higher compared with the control (Table (2) and Fig.(5)).

***Table (1)***
***Effects of L-arginine, vitamin E and their interactions on numbers and types of sperms abnormality in mice.***

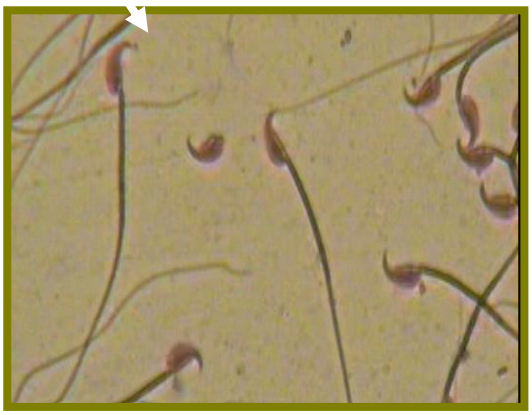
| *Treatment* | *NO. Normal sperms* | *NO. Without Head* | *NO. Without Tail* | *NO. Without Hook* | *NO. Defective Hook* | *NO. Defective Head* |
|---|---|---|---|---|---|---|
| Control | **76.64+1.70 [b]** | **8.28+1.53[b]** | **7.36+1.20[b]** | **4.68+0.21[b]** | **1.44+0.27 [a]** | **5.04+0.40 [b]** |
| L-Arginine | **73.80+3.48 [b]** | **7.40+1.19 [b]** | **6.88+1.44 [b]** | **3.14+0.39 [b]** | **0.84+0.42 [a]** | **7.72+0.94 [b]** |
| Vitamin E | **66.12+1.31 [a]** | **7.24+.97 [b]** | **8.88+1.30 [b]** | **1.00+0.38 [a]** | **1.20+0.57 [a]** | **11.04+1.63 [c]** |
| L-Arginine + Vitamin E | **96.44+0.59 [c]** | **0.80+0.141[a]** | **1.68+0.10 [a]** | **0.52+0.14 [a]** | **0.28+0.04 [a]** | **0.240+0.11 [a]** |

*Table (2)*
*Effects of L-arginine, vitamin E and their interactions on the level of LH hormone in mice.*

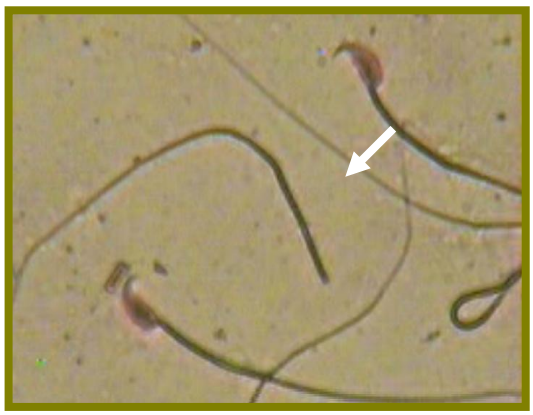
| *Treatments* | *testosterone* | *LH* |
|---|---|---|
| *Control* | 7.41±3.72 | 1.69±0.20[b] |
| *L-arginine* | 0.54±0.21 | 0.84±0.020[a] |
| *Vitamin E* | 4.87±2.98 | 0.87±0.031[a] |
| *L-arginine+ Vitamin E* | 0.19±0.04 | 1.51±0.19[b] |



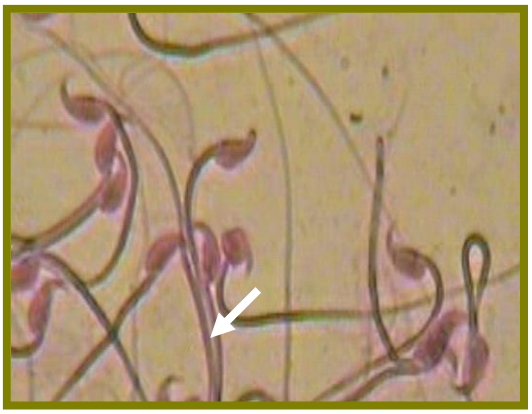
**Normal sperm**



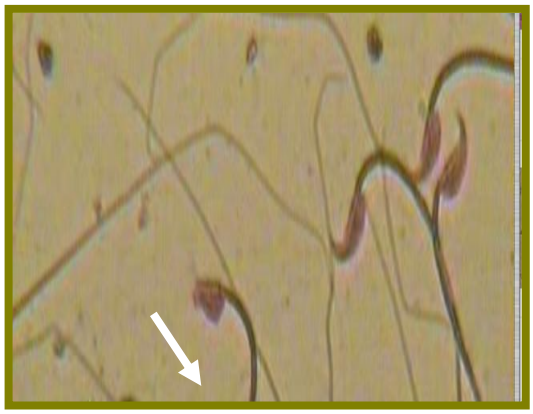
**Sperm without tail**



**Sperm without head**



**Defective head sperm**



**Sperm without hook**

*Fig.(1) Sperm abnormalities resulted from different treatments.*

## Discussion

According to the analytical values in this study, the treatment of mice with L-arginine and vitamin E alone did not decease sperm abnormality (without head and defective head), while both caused a significant increase or decrease in number of sperm abnormality (without head). Many factors lead to the the sperm abnormality especially sperm without head. Lipid peroxidation and accumulation of free radicals cause morphological damage of sperm. L-arginine which provides protection against lipid peroxidation, is less likely to act as an antioxidant (like a-tocopherol) since it does not have direct oxidizable groups. On the other hand, in neutrophils, it has been shown that the ratio of NO to superoxide determines the nature of their interaction [18]. When NO predominates, it inactivates superoxide; when superoxide predominates, it inactivates NO thus in general, higher NO concentration is expected to reduce lipid peroxidation by inactivating superoxides. As discussed before; L-arginine has been shown to increase generation of NO. Based on this, it can be postulated that L-arginine protects spermatozoa against lipid peroxidation through increased NO production. This effect may be due to antioxidant activity of these two drugs. Fat-soluble vitamins, vitamin E act as antioxidants involved in the vitamin E and it may provide a direct protection for the sperm cells from morphological damage [4]. In fact, the morphology and the motility of sperm cells would be preserved by binding of this vitamin to endoperoxides [14].

However, treatment of mice with L-arginine + vitamin E together caused a significant decease in sperm abnormality (without tail). It's known that polyunsaturated fatty acids and phospholipids are key constituents in the sperm cell membrane and highly susceptible to oxidative damage. Sperms produce controlled concentrations of reactive oxygen species, such as superoxide anion, hydrogen peroxide، and nitric oxide, which are needed for fertilization; however, high concentrations of these (free radicals can directly damage sperm cells [4]. Disruption of this delicate balance has been proposed as one of the possible etiologies of idiopathic male infertility. The high dietary levels of vitamin E may inhibit the formation of endoperoxide intermediates by preventing cyclo-oxygenase peroxidation of arachidonic acid [10].Vitamin E can alter cellular membrane structure [10] and change membrane-bound enzyme systems [12], which may result in a decreased prostaglandin formation. Our results thus support the concept that vitamin E, may reduce the minimum tissue level of peroxides needed for arachidonic acid peroxidation and ultimately reduces prostaglandin biosynthesis through its antioxidant function. Therefore, L-arginine + vitamin E together through these mechanisms have the ability to repair any disorder in sperm especially abnormal ones.

According to hook abnormality, vitamin E has very important role in improving abnormality of hook sperm except of antioxidant activity. Dietary with vitamin E has influenced directly or indirectly the spermatogenesis by non-antioxidant effects, during the second half of spermiogenesis, replacement of somatic histones by sequential expressions of spermatid nuclear transition protein (TP)-1, TP-2, and protamine results in the condensation of spermatid nuclei and initiates morphogenesis of the sperm head [6]. Heterogeneity of sperm chromatin structure and impaired DNA packaging have been linked to reduced expression of spermatid nuclear proteins [19]; [21]; [22]; and are implicated in abnormal sperm morphology [5]; [3].

Testosterone is secreted by the interstitial cells of Leydig in the testes, but only when they are stimulated by LH from the anterior pituitary gland. Furthermore, the quantity of testosterone secreted increases approximately in direct proportion to the amount of LH available [8].

The actions of testosterone and other androgens during development of sperm, exert an inhibitory feedback effect on pituitary LH secretion; develop and maintain the male secondary sex characteristics; exert an important protein-anabolic, growth-promoting effect; and, along with FSH, maintain spermatogenesis and some semi essential amino acids and alpha tocopherol have important role to homeostasis testosterone and other androgens hormone [7].

## References


[1] Adman, M. Effect of arginine on oligospermia. Fertil. Steril.. 21: 217–219. (1970)

[2] Aydin S., Inci O., Alagol, B. The role of arginine in domethacin and kallikrein in the treatment of oligospermia. Int. Urol.Nephrol. 27: 199–202. (1995).

[3] Ballachey B. E., Hohenboken W.D, Evenson D.P. Heterogeneity of sperm nuclear chromatin structure and its relationship to bull fertility. *Bio.l Reprod*. 36: 915 –925. (2001).

[4] Brzezinska-Slebodzinska, E., A. B. Slebodzinski, B. Pietras, and G. Wieczorek. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace Elem., Res., 47:69–74. (1995).

[5] Evenson, D.P., Darzynkiewicz Z., Melamed M.R. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science*. 210: 1131 –1133. (1980).

[6] Grimes, S. R., Meistrich M.L, Platz R.D, Hnilica L. S. Nuclear protein transitions in rat testis spermatids. *Expt. Cell Res*., 110: 31 –39. (1990).

[7] Ganong, W. F., (2005). Review of Medical Physiology. McGraw-Hill Companies, Inc.

[8] Guyton, A. C. and Hill J. E. Text book of Medical Physiology. 11th edition, Philadelphia, Pennsylvania. (2006). Elsevier Inc.

[9] Holt, L. E., Albanesi A. A. Observation of amino acids deficiencies in man. Trans. Assoc. Am. Physicians. 58: 143–156. (1994).

[10] Hope, W.C., Dalton, L.J. Machlin, R. J. Filipski, and F. M. Vane. Influence of dietary vitamin E on prostaglandin biosynthesis in rat blood. Prostaglandins. 10: 557-571. (1995).

[11] Kausalya S., Nath J. Interactive role of nitric oxide and superoxide anion in neutrophils-mediated endothelial cell injury. *Journal of Leukocyte Biology*. 64:185–191. (1998).

[12] Korsan-Bengtsen, K., Elmfeldt D, and Holm, T., (1974). Prolonged plasma clotting time and decreased fibrinolysis after long term treatment with alphatocopherol. Trombos. Diathes. Haemorrh. (Stuttg.). 31: 505-512.

[13] Lucy, J. A. Functional and structural aspects of biological membranes: A suggested structural role for vitamin E in the control of membrane permeability and stability. *Ann. N.Y. Acad. Sci.*, 203: 4-11. (1992).

[14] Marin-Guzman, J. D. C. Mahan, Y. K. Chung, J. L. Pate, and W. F. Pope. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality and subsequent fertilization rates in mature gilts. *J. Anim. Sci.* 75: 2994–3003. (1997).

[15] Niki, E., Yamamoto, Y., Takahashi, M., Komuro, E., Miyama, Y. Inhibition of oxidation of biomembranes by tocopherol. *Ann N Y Acad Sci.*, 570: 23-31. (1989).

[16] Patel, A. B., Srivastava, S., Phadke, R. S., Govil, G. Arginine activates glycolysis of goat epididymal spermatozoa: An NMR study. Biophys., 75: 1522–1528. (1998).

[17] Radany E. W., Atherton R. W., Forrester I. T. Arginine uptake by rabbit spermatozoa. Arch. Biochem., Biophys., 210: 770–774. (1981).

[18] Scibona, M., Meschini, P., Capparelli, S., Larginine and male infertility. Minerva Urol Nefrol, 46:251-253. (1994).

[19] Steger, K., Failing K., Klonisch, T., Behre, HM, Manning, M., Weidner, W., Hertle, L., Bergmann, M., Kliesch, S., Round spermatids from infertile men exhibit decreased protamine-1 and -2 mRNA. *Hum Reprod*. 16: 709 –716. (2001).

[20] Verma A., Kanwar KC Effect of vitamin E on human sperm motility and lipid peroxidation *in vitro*. Asian J. Androl. 1: 151-4. (1999).

[21] Yu, Y. E., Zhang, Y., Unni, E., Shirley C.R., Deng, J.M., Russell, L.D., Weil, M.M., Behringer, R.R., Marvin, L., Meistrich, M.L. Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. *Proc Natl Acad Sci U S A*., 97: 4683 – 4688. (2000).

[22] Zhao M., Shirley, C.R., Yu, Y. E., Mohapatra, B., Zhang, Y., Unni, E., Deng J.M., Arango N.A., Terry, N.H.A., Weil,

M.M., Russell, L.D., Behringer, R. R. and Meistrich, M.L., Targeted disruption of the transition protein 2 genes affects sperm chromatin structure and reduces fertility in mice. *Mol Cell Biol*., 21: 7243 –7255. (2001).

**الخلاصة**

صممت الدراسة لتقييم تأثير L-Arginine، فيتامين E وتداخلاتهما فى مورفولوجي الحيامن (نوعية الحيامن). تضمنت الدراسة عشرون ذكرا من الفئران البيضاء قسمت الى أربعة مجاميع (مجموعة السيطرة، مجموعة عوملت بفيتامين E، مجموعة عوملت بـــ L-Arginin اما المجموعة الأخيرة فقد عوملت بفيتامين E مع L-Arginine). وضعت حيوانات التجربة تحت ظروف مناسبة من حرارة ( 24± 2 درجة مئوية) وفترة ضوئية (12-14) ساعة/يوم.

اظهرت النتائج بان فئة الفئران المعاملة بفيتامين E مع L-Arginine (0.8 ± 0.141) كان عدد الحيامن المشوهة فيها (بلا رأس وبلا ذيل) معنويا ( $P \leq 0.05$) اقل عددا من الحيامن المشوهة في فئة السيطرة.

وصلت اعداد الحيامن المشوهة بدون ذيل (1.68±0.1) و الفئران المعاملة بفيتامين E (1± 0.38) بالاضافة الى فيتامين E مع L-Arginine ( 0.52 ± 14) وأدت الى انخفاض معنوي في الحيامن بدون كلاب. بينما الفئران المعاملة بفيتامين E فقط سببت انخفاضا معنويا فى أعداد الحيامن المشوهة بدون رأس مقارنة مع مجموعة فئران السيطرة. عوملت جميع الفئات لمدة ثلاثون يوم. تم حساب النسبة المئوية لكل فئة تمت دراستها باستخدام مائتي حيوان منوي.