

# Evaluation of androgenic activity of allium cepa on spermatogenesis in the rat

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*Allium cepa* (onion) has a beneficial effect on disease treatment worldwide and has been used since ancient times as a medicinal and food source. Recently several reports have shown that onion has high antioxidant activity. As antioxidants have an essential effect on sperm health parameters, we investigated the effect of the fresh juice of onion bulbs on the spermatogenesis cycle in rats. Wistar male rats ( $n = 30$ ) were allocated into 3 groups, control ( $n = 10$ ) and two test groups (each of 10). The animals in the test groups were subdivided into groups of 2 that received fresh onion juice equivalent to 0.5 and 1 g/rat/day of fresh onion. The fresh onion juice was administered by gavage for 20 consecutive days. The animals were kept in standard conditions. On the twentieth day, the testes of rats in all groups were removed and sperm was collected from the epididymis and was prepared for analysis.

Serum total testosterone significantly increased in all the test groups ( $p < 0.05$ ), and levels of LH significantly increased only in the group that received the high dose of fresh onion juice ( $p < 0.05$ ), but the level of FSH did not differ between the experimental and control groups. The percentage of sperm viability and motility in both test groups significantly increased ( $p < 0.05$ ), but the sperm concentration significantly increased only in the group that received the high dose of freshly extracted onion juice ( $p < 0.05$ ). It was evident that there was no difference on sperm morphology and testis weight in test groups compared to the control group.

In our study, freshly prepared onion juice significantly affected the sperm number, percentage of viability, and motility; it seems that using 4 g/kg of freshly prepared onion juice is effective in sperm health parameters. (Folia Morphol 2009; 68, 1: 45–51)

**Key words:** allium cepa, infertility, spermatogenesis

## INTRODUCTION

In the last few years, a marked decrease in the quality of semen has been reported [3]. Infertility is one of the major health problems in couples' lives; approximately 30% of couples infertility is due to male factors [13]. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution, and insufficient vitamin intake may have harmful effects on spermatogenesis and the normal production of sperm [26]. Researchers have reported that using antioxidants and vitamins A, B, C, and E in the daily diet can protect sperm DNA from free radicals and increase blood testis barrier stability [14, 20]. The onion (*Allium cepa*) has long been used in traditional medicine, is one of the important *Allium* species commonly used in our daily diet, and has recently been the source of much interest because of its antithrombotic, hypolipidaemic, hypotensive, diaphoretic, antibiotic, antidiabetic, antiatherogenic, and anticancer medicinal properties [1, 18]. The biological action of *Allium* products is ascribed to organosulfur and phenolic compounds. It has been found that administration of onion products to diabetic rats significantly reduced hyperglycaemia [17]. Furthermore, the role of nutritional factors in reproduction and subfertility is important. Research has shown that onion contains exogenous and endogenous antioxidants such as selenium, glutathione, vitamins A, B, and C, and flavonoids such as quercetin and isorhamnetin [22]. These antioxidants protect DNA and other important molecules from oxidation and damage, which would otherwise induce apoptosis, and could improve sperm health parameters, increasing the rate of fertility in men [29, 35]. The aim of the present study was to evaluate the androgenic effects of different doses of onion bulb juice on sperm parameters by using hormone measurements and histopathological studies.

## MATERIAL AND METHOD

### Plant material

#### *Preparation of onion juice*

The underground yellowish-white bulbs of *Allium cepa* (onion) was collected in August 2007 from Ilkhchi in the province of East Azerbaijan-Iran. The skin was removed and fresh juice of onions was prepared using a Tefal fruit juice extracting machine before the experiments.

#### *Analysis of onion juice*

The onion juice was tested for the determination of flavonoids using the Shinoda test [37]. Qualitative thin-layer chromatography (TLC) was employed for determination of quercetin as a main flavonoid in onion. For TLC, 10 mL of fresh onion juice was dried in a vacuum and the resulting residue dissolved in 1 ml of methanol. 20  $\mu$ L of methanolic solution was spotted on a silica gel plate (10  $\times$  20 cm, silica gel 60 GF254, Merck, Darmstadt, Germany) with a solvent system of EtOAc/MeOH (80:20). Quercetin, Sigma chemical Co. (St. Louis, MO, USA) was used as a control. After developing and drying, the TLC plate was sprayed with a 2% AlCl<sub>3</sub> solution in methanol. Quercetin in the onion samples appeared as a yellow spot at RF = 0.6. Separation of quercetin was performed with further purification by preparative TLC on silica gel and quantitative determination of quercetin carried out on a Model 2100 Spectrophotometer (Shimadzu, Japan) in 370 nm comparing to a pure quercetin standard curve. The amount of quercetin in fresh onion was 12 mg/100 g.

#### Experimental animals

The 30 adult Wistar albino male rats were 8 weeks old and weighed 250  $\pm$  10 g, they were obtained from the animal facility of the Pasture Institute of Iran. The male rats were housed in temperature controlled rooms (25°C) with constant humidity (40–70%) and 12 h/12 h light/dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz Medical University.

All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to the start of treatment, in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly selected and divided into control (n = 10) and experimental (n = 20) groups. The experimental groups were divided into two groups of ten. One of these groups received 0.5 g/rat and the other received 1 g/rat of fresh onion juice by gavage for 20 consecutive days. The control group just received 1cc of distilled water by gavage.

#### Surgical procedure

On the twentieth day (at the end of the treatment period) the rats were killed with diethyl ether,

and the testes in the control and experimental groups were immediately removed. The weight of the testes in all study groups was recorded.

#### **Epididymal sperm motility, viability, and counts, and sperm abnormality**

Sperm from the cauda epididymis were released by cutting into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin [15]. After 5 min incubation at 37°C (with 5% CO<sub>2</sub>), the cauda epididymis sperm reserves were determined using the standard haemocytometric method, and sperm motility was analyzed by microscope (Olympus IX70) at 10 field and reported as the mean of motile sperm according to WHO methods [34]. The sperm abnormality was evaluated according to the standard method of Narayana [27]. Briefly, smears of the sperm suspension were made on clean glass slides and stained with periodic acid-Schiff's reaction haematoxylin. The stained smears were observed under a light microscopic with 40× magnification. The sperms were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence.

#### **Serum total testosterone, LH, and FSH hormone**

Serum concentration of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA) Rat FSH/LH kits obtained from Biocode, Belgium, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2 ng/mL and 0.14 ng/mL for FSH and LH, respectively. Serum concentration of total testosterone was measured by using a double antibody RIA kit from Immunotech Beckman Coulter, USA. The sensitivities of hormone detected per assay tube were 0.025 ng/mL [12].

#### **Total antioxidant capacity and malondialdehyde concentration**

##### *Measurement in serum*

A total antioxidant capacity (TAC) detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute, China. According to this method, the antioxidant defence system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>. TAC was measured by the reaction of phenanthroline and Fe<sup>2+</sup> using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidant required to

make absorbance increase by 0.01 in 1 mL of serum [9]. Free radical damage was determined by specifically measuring malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a coloured product that was measured at 532 nm (MDA detecting kit from Nanjing Jiancheng Bioengineering Institute, China) [28].

##### *Histology*

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron-thick sections were prepared and stained with haematoxylin and eosin (HE). The specimens were examined under an Olympus/3H light microscope. The diameter of the seminiferous tubules was measured in 20 round tubular sections per animal at 100× magnification and the digitized images were analyzed for morphometric study. Then *D* — mean diameter, *a* — high diameter, and *b* — low diameter were measured and substituted (as *a* and *b*) in this formula:  $D = \sqrt{a \times b}$ . The software for the measurement of the diameters of seminiferous tubules was Image Toll 2007 [15].

#### **Statistical analysis**

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± SEM (standard error of means). Significant difference is written in parentheses.

## **RESULTS**

#### **Weight of individual male testis**

There was no significant change in testis weight between the control and experimental groups (Table 1).

#### **Sperm motility, viability, count, and abnormality**

Administration of 0.5 g/rat and 1 g/rat of freshly prepared onion juice for 20 consecutive days significantly increased sperm motility and viability in both experimental groups as compared to the control group, using the Dunnett homogeneity test (Table 1). Sperm count was significantly increased in the experimental group that received 1 g/kg freshly prepared onion juice as compared with the control group (Table 1). Sperm abnormality was not significantly different in the experimental group that received 1 g/kg freshly prepared onion juice, compared with the control group (Table 1).

**Table 1.** The effect of 0.5 g/rat and 1 g/rat of onion on sperm parameters, serum FSH, LH, total testosterone, testes weight, and diameter of seminiferous tubules of control and experimental groups in rats

	Control (n = 10)	Onion (0.5 g/kg-per day) (n=10)	Onion (1 g/kg-per day) (n = 10)
Testis [g]	1.40 ± 0.821	1.26 ± 0.355	1.42 ± 0.829
Sperm concentration (total count) (No. of sperm/rat × 10 <sup>6</sup> )	48.68 ± 7.70 (0.319)	57.35 ± 5.36 (0.219)	75.70 ± 2.34* (0.008)
Motility (%)	33.75 ± 6.88 (0.112)	73 ± 9.43* (0.003)	87 ± 5.14* (0.002)
Viability (%)	66.25 ± 4.73 (0.281)	93.60 ± 1.83* (0.006)	96.20 ± 1.20* (0.009)
Diameter of seminiferous tubules [μm]	380.1 ± 0.1	377.3 ± 0.1	385.5 ± 0.1
Serum testosterone levels [ng/mL]	1.60 ± 0.091 (0.189)	3.908 ± 0.221* (0.004)	5.017 ± 0.949* (0.003)
Sperm abnormality (%)	6.27 ± 0.711	5.20 ± 0.618	5.12 ± 0.656
LH levels [ng/mL]	1.51 ± 0.138 (0.371)	2.01 ± 0.197 (0.315)	2.94 ± 0.473* (0.002)
FSH levels [ng/mL]	20.37 ± 1.788	23.49 ± 1.894	26.61 ± 1.597
Total antioxidant capacity (TAC)	0.53 ± 0.777 (0.151)	0.53 ± 0.138 (0.211)	0.88 ± 0.033* (0.001)
Malondialdehyde (MDA)	4.80 ± 0.212 (0.105)	3.30 ± 0.228* (0.002)	2.43 ± 0.182* (0.006)

Data are presented as mean ± SE; significant different at writing in parentheses (compared with the control group, using Dunnett homogeneity test). The significant different is very low. In testis, diameter of seminiferous tubules, sperm abnormality, and FSH levels, all groups equalled, respectively, with significant different: 0.491, 0.381, 0.467, and 0.501.

### Serum total testosterone, LH, and FSH hormone measurement

Administration of 0.5 g/rat and 1 g/rat of fresh onion juice daily for 20 consecutive days significantly increased serum total testosterone. Administration of 0.5 g/rat of fresh onion juice daily for 20 consecutive days could not increase the LH hormone level but 1 g/rat fresh onion juice could increase LH hormone level in the experimental group compared to the control group. There was no significant difference in the level of FSH hormone between the experimental and control groups (Table 1).

### Total antioxidant capacity and malondialdehyde concentration

#### Measurement in serum

Administration of 0.5 g/rat and 1 g/rat of fresh onion juice daily for 20 consecutive days significantly decreased the level of MDA concentration in the experimental groups compared to the control group ( $p < 0.05$ ). Administration of 1 g/rat of fresh onion juice daily for 20 consecutive days could have significantly increased the level of TAC. However, 0.5 g/rat

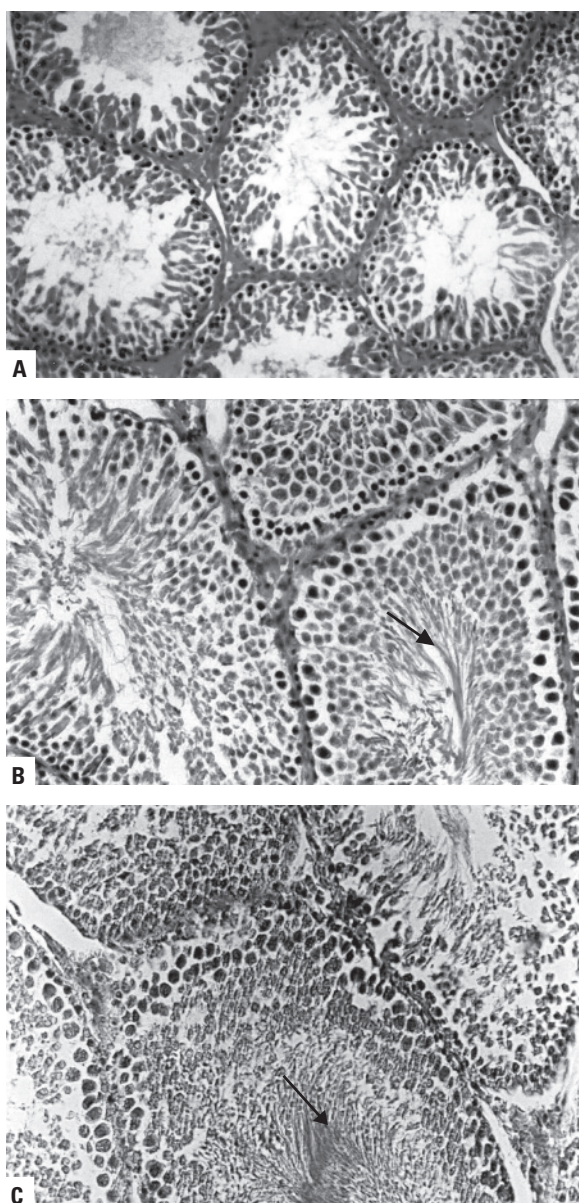
of fresh onion juice did not have any significant effect on TAC in the experimental groups (Table 1).

#### Histology

The histopathological study showed the cycle of spermatogenesis was regular in all experimental and control groups (Fig. 1A), but there was no significant difference in seminiferous tubules between the control group and the group that received 0.5 g/rat of fresh onion juice (Fig. 1B). However, in all animals exposed to 1 g/rat of fresh onion juice, an accumulation of sperm in the lumen of the seminiferous tubules was observed (Fig. 1C). The diameter of the seminiferous tubules showed no significant difference in any animals exposed to 0.5 g/rat and 1 g/rat of fresh onion juice, as compared with those measured in the control group (Table 1).

## DISCUSSION

Infertility is one of the major problems in couples' lives; about 25% and 35% of infertility is attributed to the male and the female's receptivity, respectively [3]. Many environmental and biochemical factors are involved in male and female repro-



**Figure 1.** **A.** Regular seminiferous tubule with normal germinal epithelium morphology in the control group (H&E,  $\times 320$ ); **B.** Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence. In lumen of seminiferous tubule (arrow) in 0.5 g/rat fresh onion juice group (H&E,  $\times 640$ ); **C.** Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence. In lumen of seminiferous tubule (arrow) in 1 g/rat fresh onion juice group (H&E,  $\times 640$ ).

duction [26]. The importance of many of these factors is not yet clearly understood. A better understanding of the underlying mechanisms in (sub) fertility and better study results clarifying the effectiveness of nutritional and biochemical factors are important to improve diagnosis and treatment. Smart choices with regard to a better diet might protect the body from many diseases [5]. The main

advice for a healthy diet is to eat more fruit and vegetables. However, published intervention trials do not yet support this message [2, 32]. Onion and garlic contain a wide variety of phytochemicals and micro constituents such as trace elements, vitamins, fructans, flavonoids, and sulphur compounds, which may have a protective effect against free radicals. Recently, much attention has been focused on the protective effects of onion against colon cancers in rats [10, 30]. The present results clearly indicate that *Allium cepa* (onion) has a good effect on spermatogenesis in rats. Our results showed that administration of onion juice (1 g/rat/day) for 20 consecutive days caused a marked increase in sperm count, viability, and motility, as compared to respective controls. These effects could be related to vitamins, vitamin C, and flavonoids of onion such as quercetin. Oxidative damage was ascertained by measuring malondialdehyde levels, reactive oxygen species (ROS) generation, alterations in antioxidant defences, and the extent of protein oxidation. Quercetin, an important flavonoid, has a beneficial effect on health due to its antioxidant function. One mechanism of the antioxidant action of quercetin is involved in scavenging free radicals such as superoxide radicals generated by xanthine/ xanthine oxidase [7]. Studies on the effect of quercetin on oxidative damage in cultured chicken spermatogonial cells showed quercetin to have no deleterious effect on spermatogonial cells at doses of 1  $\mu\text{g/mL}$  and 10  $\mu\text{g/mL}$ . Quercetin (1  $\mu\text{g/mL}$ ) increased the number of spermatogonial cells and decreased the mortality of Aroclor-induced oxidative damage. In this study, the effect of quercetin on serum MDA was determined, but the results indicated no obvious effect of quercetin on MDA production [24, 25]. Vitamins C and E are well known antioxidants that can ameliorate oxidative stress-related testicular impairments in animal tissues [8, 11, 16, 21]. Vitamin C may execute its role by modulating testicular free radical production and/or stimulating testicular androgenesis and is essential for testicular differentiation, integrity, and steroidogenic functions [6, 19, 31, 33]. Furthermore, vitamin C is an antioxidant in semen and thus protects sperm from oxidative damage [4, 36, 37]. Meeker and co-workers found that FSH, LH, inhibin-B, testosterone, and free T4 levels were associated with human semen parameters. They showed that serum levels of FSH and LH are inversely associated with sperm concentration, motility, and morphology. FSH, which is a gonadotropin that is produced and secreted by the anterior pituitary, acts

on Sertoli cells in the seminiferous tubules to initiate spermatogenesis. Sertoli cells secrete inhibin-B, which is a protein hormone. The inverse associations of FSH, with inhibin-B and with sperm concentration, may be due to the feedback effects exerted by inhibin-B on the anterior pituitary to inhibit FSH secretion. The results suggest that FSH, LH, and inhibin-B play a role in sperm development (morphology) increasing levels of FSH and LH, but decreasing levels of inhibin-B, and also suggest that FSH, LH, and testosterone have an impact on sperm motion (motility). Testosterone increases sperm motility and LH decreases sperm concentrations, motility, and morphology [23]. Our data showed serum total testosterone significantly increased in test groups ( $p < 0.05$ ) and levels of LH significantly increased only in the group that received a high dose of fresh onion juice ( $p < 0.05$ ), but there was no difference in the level of FSH between the experimental and control groups. The percentage of sperm viability and motility in the test groups significantly increased ( $p < 0.05$ ), but the sperm concentration significantly increased only in the group that received the high dose of fresh onion bulb juice, ( $p < 0.05$ ) but had no effect on sperm morphology and testis weight in both groups compared to the control group. Thus, it seems that using 1 g/kg of fresh onion juice can be effective for healthy sperm parameters. The results revealed that MDA decreased and TAC increased with onion juice.

These alterations could be due to the vitamin and quercetin content of onion. Therefore, these results indicated that antioxidants and vitamins from foods consumed by animals, such as quercetin, vitamin C, vitamin B, and vitamin E, could improve sperm health parameters and testicular androgenesis.

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### REFERENCES

- Augusti KT (1996) Therapeutic values of onion and garlic. *Indian J Exp Biol*, 34: 634–640.
- Beresford SA, Johnson KC, Ritenbaugh C (2006) Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA*, 295: 643–654.
- Carlsen E, Giwercman A, Keiding N, Skakkebaek NE (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ*, 12: 609–613.
- Castllini C, Lattaioli P, Dal BA, Minelli A, Mugnai C (2003) Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Reprod Nutr Dev*, 43: 91–103.
- Cummings JH, Bingham SA (1998) Fortnightly review — diet and the prevention of cancer. *Br Med J*, 317: 1636–1640.
- Dawson EB, Harris WA, Powell LC (1990) Relationship between ascorbic acid and male fertility. *World Rev Nutr Diet*, 62: 1–26.
- Dok-Go H, Lee KH, Kim HJ, Lee EH, Lee J, Song YS, Lee YH, Jin C, Lee YS, Cho J (2003) Neuroprotective effects of antioxidative flavonoids, quercetin, (1) dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var. *saboten*. *Brain Res*, 965: 130–136.
- El-Missiry MA (1999) Enhanced testicular antioxidant system by ascorbic acid in alloxan diabetic rats. *Comp Biochem Phys*, 124: 233–237.
- Feng R, He W, Ochi H (2001) A new murine oxidative stress model associated with senescence. *Mech Ageing Dev*, 122: 547–559.
- Fukushima S, Takada N, Hori T, Wanibuchi H (1997) Cancer prevention by organosulfur compounds from garlic and onion. *J Cell Biochem*, 27: 100–105.
- Ghosh D, Das UB, Misro M (2002) Protective role of alpha-tocopherol-succinate (provitamin-E) in cyclophosphamide induced testicular gametogenic steroidogenic disorders: a correlative approach to oxidative stress. *Free Radic Res*, 36: 1199–208.
- Huang HFS, Linsenmeyer TA, Li MT, Giglio W, Anesetti R, von Hagen J, Ottenweller JE, Pogach L (1995) Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study. *J Androl*, 16: 148–157.
- Isidori AM, Pozza C, Gianfrilli D, Isidori A (2006) Medical treatment to improve sperm quality. *J Reprod Biomed*, 12: 704–714.
- Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B, Penkowski A (2006) Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. *J Reprod Dev*, 52: 203–209.
- Khaki A, Ghaffari Novin M, Khaki AA, Nouri M, Sanati E, Nikmanesh M (2008) Comparative study of the effects of gentamicin, neomycin, streptomycin and ofloxacin antibiotics on sperm parameters and testis apoptosis in rats. *Pakist J Biol Scien*, 11: 1683–1689.
- Kujo S (2004) Vitamin C: basic metabolism and its function as an index of oxidative stress. *Curr Med Chem*, 11: 1041–1064.
- Kumud K, Biju CM, Augusti KT (1990) Antidiabetic and hypolipidemic effects of SMCS isolated from *Allium Cepa* Linn. *Indian J Biochem Biophys*, 32: 49.
- Lee SU, Lee JH, Choi SH, Lee JS, Ohnisi-Kameyama M, Kozukue N, Levin CE, Friedman M (2008) Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial onion products. *J Agricult Food Chem*, 24: 8541–8548.
- Luck MR, Jeyaseelan I, Scholes RA (1995) Ascorbic acid and fertility. *Biol Reprod*, 52: 262–266.

20. Lu P, Lai BS, Liang P, Chen ZT, Shun SQ (2003) Antioxidation activity and protective effect of ginger oil on DNA damage in vitro. *J Zhongguo Zhong Yao Za Zhi*, 28: 873–875.
21. Marchlewicz M, Wiszniewska B, Baranowska-Bosiacka I, Safranow K, Kolasa A, Glabivski W (2007) Increased lipid peroxidation and ascorbic acid utilization in testes and epididymis of rats chronically exposed to lead. *Biometals*, 20: 13–19.
22. Markham KR (1982) Techniques of flavonoid identification. Academic Press, London.
23. Meeker JD, Godfrey-Bailey L, Hauser R (2007) Relationships between serum hormone levels and semen quality among men from an infertility clinic. *J Androl*, 28: 397–406.
24. Mi Y, Zhang C (2005) Protective effect of quercetin on Aroclor 1254-induced oxidative damage in cultured chicken spermatogonial cells. *Toxicol Scien*, 88: 545–550.
25. Mi Y, Zhang C, Taya K (2007) Quercetin protects spermatogonial cells from 2,4-d-induced oxidative damage in embryonic chickens. *J Reprod Dev*, 53: 749–754.
26. Mosher WD, Pratt WF (1991) Fecundity and infertility in the United States: incidence and trends. *J Fertil Steril*, 56: 192–193.
27. Narayana K (2008) An aminoglycoside antibiotic gentamycin induces oxidative stress, reduces antioxidant reserve, and impairs spermatogenesis in rats. *J Toxicol Sci*, 33: 85–96.
28. Quintanilha AT, Packer L, Davies JM, Racanelli TL, Davies KJ (1982) Membrane effects of vitamin E deficiency: bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. *Ann NY Acad Sci*, 393: 32–47.
29. Rajeev K, Gagan G, Narmada P (2006) Drug therapy for idiopathic male infertility: rationale versus evidence. *J Urology*, 176: 1307–1312.
30. Ross SA, Finley JW, Milner JA (2006) Allyl sulfur compounds from garlic modulate aberrant crypt formation. *J Nutr*, 136: 852S–854S.
31. Salem MH, Kamel KI, Yousef MI, Hassan GA, EL-Nouty FD (2001) Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B1. *Toxicology*, 162: 209–218.
32. Schatzkin A, Lanza E, Corle D (2000) Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. *New Engl J Med*, 342: 1149–1155.
33. Thews O, Lambert C, Kelleher DK, Biesalski HK, Vaupel P, Frank J (2005) Possible protective effects of alpha-tocopherol on enhanced induction of reactive oxygen species by 2-methoxyestradiol in tumors. *Adv Exp Med Biol*, 566: 349–355.
34. World Health Organization (1999) WHO Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 4<sup>th</sup> Ed. Cambridge University Press, New York.
35. Yang HS, Han DK, Kim JR, Sim JC (2006) Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. *J Korean Med Sci*, 21: 445–451.
36. Yousef MI, Abdallah GA, Kamel KI (2003) Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Anim Reprod Sci*, 76: 99–111.
37. Yousef MI (2005) Protective effect of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. *Toxicology*, 207: 81–89.