

Case study

The Influence of Nutrition on Quality of Semen and Stamina Case Study: Vitolize and Bee Pollen nutritional products

Abstract

Several researches have focused on male infertility as an essential concern of man-life discontinuity. Roughly, 30-80% of infertility cases are because of oxidative stress and declined level of seminal total antioxidant capacity. There is few information about the influence of nutrition on quality of semen. The objective of this study was to detect the effects of nutritional products Vitolize and Bee pollen, which contain vitamin C and the B vitamins and other supplements for improving major semen parameters such as sperm concentration, motility, and morphology and fertility rate. A semen sample was obtained from a 42 years a male donor facing fertility problems. Analysed before and after treatment with the products in Queens laboratory, Nemra Talata. The results of this study indicated that after treatment, there was a significantly increase in sperm count, and an increase in progressive motility from 58.428 to 70.53 million/ejaculate, and 7.17 to 14.29 % respectively. There was an increase in the number of live sperm cells, from 3 to 7 sperms. The current study also indicated that the products supplements for Vitolize and Bee pollen especially a combination of antioxidants such as vitamin C, vitamin E, and other supplements intake can effectively improve semen parameters in infertile men.

Key words: *Influence Nutrition, Quality, Semen.*

Introduction

Barrenness can be defined as not being able to get the women into pregnancy state despite having continues, unprotected sex for at least 12 months (1). More than 70 million couples facing infertility globally. Male infertility is an essential concern throughout the world. Between 8 and 12% of couples suffer from barrenness, based on research (2). Male factors account for at least 50% of all infertility cases worldwide (3). Some factors such as radiation, smoking, varicocele, infection, urinary tract infection, environmental factors, nutritional deficiencies and oxidative stress contribute to male infertility (4, 5). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's natural antioxidant defenses (6).

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Comment [s2]: The referred study says that most studies reported 14% of infertility in the couples. There is a need to verify all the references.

Comment [s3]: Genetic factors can also be one of the reason for infertility. Due to Chromosome defects, chromosome abnormalities are found in which there is Deletion or duplication of whole chromosomes and Inversion, or duplication of a portion of a chromosome or there is translocation of part of a chromosome to another Chromosome.

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These increased level of ROS can be resulted from environmental factors such as high temperature, electromagnetic waves, air pollution, insecticides, alcohol consumption, obesity and poor nutrition (7). There are evidence that sperms are simply affected by ROS and oxidative stress.

There are several researches that assist the role of ROS in male infertility theory (8-9). Products such as vitolize and bee pollen can introduce a high improvement for quality of semen, because they contain essential supplements such as Vitamins C and E. Ordinary antioxidants in semen include vitamin E, vitamin C, superoxide dismutase, glutathione and thioredoxin. These antioxidants neutralize free radical activity and protect sperm from ROS that already produced (6).

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Materials and Methods

Study Area

The research was done in Queens Specialist private hospital, Queens Specialist private hospital is located around kokora road Juba, in Nimara Talata South Sudan. The hospital has simpler team to assist it giving faster, more useful care to patients and their families with an enhanced workflow.

Sample Collection

The sample was collected after 30 days and 4 days of abstinence, before and after treatment respectively from a 42 years adult, and then was taken to the laboratory for analysis.

Treatment prescription

The doses were applied as follows:

For Vitolize product, 1×2/ 30 days and for Bee pollen, 2 in morning and 1 in the evening/30days

Sample Analysis

Semen samples were collected at the hospital by masturbation into a sterile plastic container. The samples were liquefied for 30 min in 37 °C before analysis. Macroscopic examination of semen was performed according to the 5th edition of WHO laboratory manual for the examination and processing of human semen (61). Microscopic measurements of the sperm count, concentration, motility and morphology were determined with the use of computer-

aided semen analysis (CASA). The basic components of the system were a bright field microscope (Olympus CX41, Tokyo, Japan), a digital camera to capture images (Olympus U-CMAD3), and a computer with software installed (SCA@Microptic S.L., Barcelona, Spain). The WHO (61) cut-off points were used to evaluate abnormal values of semen quality parameters (Table 1). All analyses were performed by an experienced technician.

Table 1. Sperm Physical properties before and after treatment with Vitolize nutritional product

<i>Property</i>	<i>Before treatment</i>	<i>.After Treatment</i>	<i>Standard range</i>
<i>Abstinence</i>	30 Days	4 Days	2-5 Days
<i>Collection</i>	Masturbation at lab	Masturbation at lab	
<i>Volume</i>	1.8 ml	4 ml	>=1.5 ml
<i>PH</i>	7.5	8	>=7.2
<i>Colour</i>	Grey Opalescent	Grey Opalescent	
<i>Viscosity</i>	Moderate	Moderate	
<i>Liquefaction time</i>	30 Min	50 Min	15 - 60 min.
<i>Liquefaction State</i>	Complete	Complete	

Results

Results for semen analysis, sperm count and progressive motility

The results for semen analysis is illustrated in table 2.

Table 2. Test results for semen analysis, sperm count and progressive motility

<i>Test</i>	<i>Before treatment with Vitolize nutritional product Results</i>	<i>After treatment with nutritional product Results</i>	<i>Status</i>	<i>Standard Range</i>
<i>Concentration</i>	32.46	23.51	passed	≥ 15 million/ml
<i>Total sperm count</i>	58.428	70.53	passed	≥ 39 million/ejaculate
<i>Progressive motility(PR)</i>	7.17	14.29	failed	$\geq 32\%$
<i>Rapid PR</i>	0.00	0.0		
<i>Slow PR</i>	7.17	14.29		
<i>Total motility (PR+NP)</i>	22.88	19.05	failed	$\geq 40\%$
<i>Vitality</i>	60	87.5	passed	$\geq 58\%$

The results for semen analysis showed that after treatment there was an increase in sperm count, and progressive motility from 58.428 to 70.53 million/ejaculate, and 7.17 to 14.29 % respectively (Table.2) and (Figure1.).

Results for progressive motility

Results for progressive motility is represented in Figure 1.

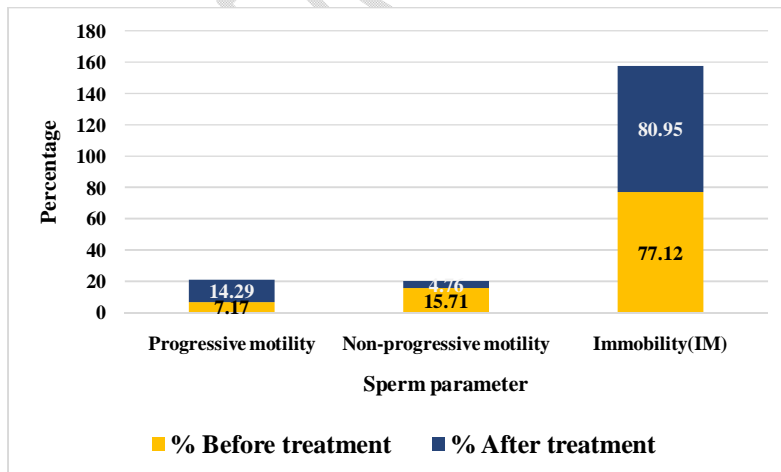


Figure 1. Progressive motility

Results for semen analysis, the dead and live sperm estimation

The results for semen analysis, the dead and live sperm estimation is represented in Figure 2.

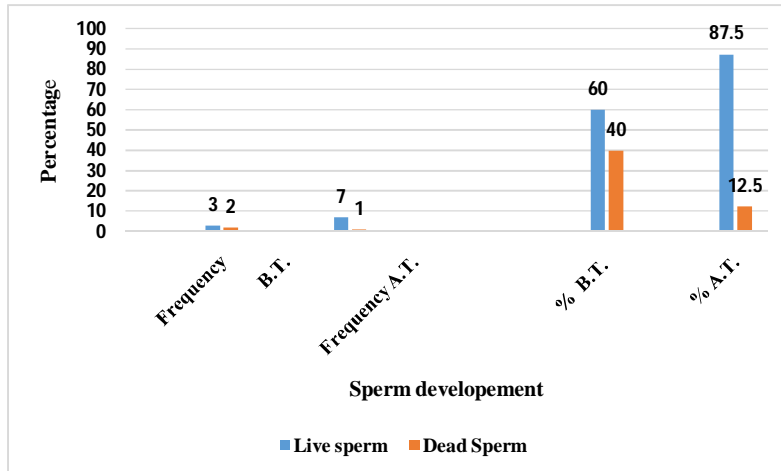


Figure 2. Semen analysis, the dead and live sperm estimation

The results showed that there was an increase in the number of live sperm cells, from 3 to 7 sperms (Figure2.)

Discussion

It is believed that oxidative stress significantly affects male infertility. The results of this indicated that after treatment there was an increase in sperm count, and progressive motility from 58.428 to 70.53 million/ejaculate, and 7.17 to 14.29 % respectively (Table.2) and (Figure1). And that there was an increase in the number of live sperm cells, from 3 to 7 sperms (Figure2.)

The results are all in line with other previous studies that focused on the effect of nutrition on sperm key parameters, their results showed a positive relationship between nutrition and semen parameters such as sperm count, motility and morphology (15, 19, and 35). One study indicated no significant relationship between nutrition therapy and semen parameters according to the smaller sample size compared to other researches (20). A meta-analysis by Lafuente indicated that treatment with coenzyme Q10 led to a significant improvement in the sperm motility and density, whereas no significant improvements was observed in live birth and pregnancy rates (8). The combination of vitamin E and vitamin C indicated no improvement in either sperm count or motility, but reduced sperm DNA damage (12).

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Several studies have indicated that combined selenium and N-acetyl-cysteine therapy improved male infertility (31-32). Moslemi found that combination therapy with selenium and vitamin E significantly improved sperm motility and morphology. In spite of, the sample size was large, this study lacked the control group (13). Two studies indicated that no improvement in sperm motility and morphology after zinc therapy (28, 29). However, two other studies showed a significant improvement in zinc-attached ligands and semen key enzymes after zinc therapy (26, 30).

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All studies which tested the influence of multiple antioxidants in a supplementation showed an improvement in semen parameters after therapy (34, 35). For example, combination therapy with carnitine, CoQ10, vitamin E and vitamin C for three to six months improved sperm concentration (36). Only one study indicated a significant improvement in sperm concentration after combination therapy without enhancement in motility and morphology (33).

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Conclusion

In conclusion, in spite of several of reviewed researches indicated significant relation between antioxidant supplementations and one or two semen parameters but application of supplementations such as L-carnitine, selenium, vitamin C and vitamin E may contribute to enhancing sperm concentration, motility and morphology.

Recommendations

In order to maintain and increase the sperm vitality, mobility, concentration and mobility, the nutritional products such as Vitolize and Bee pollen should be taken regularly, at least once every six month because they contain most of the following (Table.3):

Table 3. BFCs and their major outcomes on human sperm quality and function.

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Nutritional Factor	Major Outcomes	References
Vitamin A	- Normal blood-testis barrier function; - Avoids germ-cell aplasia; - Fertile men have higher serum concentrations than infertile.	(36)
Vitamin C	- Improved sperm cell count, motility, and morphology; - Lower levels of vitamin C in seminal plasma of infertile men.	(37,38)
Vitamin E	- Higher live-birth rate, and a trend of better results of in vitro fertilization parameters; - Decreases the lipid peroxidation of the sperm cell and seminal plasma; - Improves sperm cell motility;	(36,39,40)

	- Lower levels were found in men with oligozoospermia and asthenozoospermia.	
Vitamin D	- The expression of vitamin D receptors and metabolizing enzymes are marked in human testis, ejaculatory tract, and mature sperm cells; - Positive association between serum levels and sperm motility;	(41-43)
Vitamin B9	Protects against DNA damage.	(44,45)
Selenium	- Protects against ROS; - Deficiency promotes sperm cell abnormalities, and affects motility and fertility;	(46,47)
Zinc	- Important for spermatogenesis: cofactor of enzymes involved in DNA transcription and protein synthesis; - Lower zinc levels in the seminal plasma of infertile men; - Increased the normal sperm cell morphology, sperm motility, and semen volume.	(48,49)
N-acetylcysteine	- Improved the volume, motility, and viscosity of sperm cells; - Increased the serum total antioxidant capacity; - Reduced the serum peroxide and oxidative stress; - Increased sperm cell concentration, motility, and percent normal morphology in infertile men.	(47,50)

References

1. Brugh VM, Lipshultz LI. Male factor infertility: evaluation and management. *Med Clin North Am*; 2004. 88: 367-385.
2. Irvine DS. Epidemiology and aetiology of male infertility. *Hum Reprod* 13 (suppl.): .1998. 33-44.
3. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ. Best practice policies for male infertility. *Fertil Steril*; .2002. 77: 873-882.
4. Olayemi F. review on some causes of male infertility. *Afr J Biotech* 9. 2010.
5. Wong WY, Thomas CM, Merkus JM, Zielhuis GA, Steegers-Theunissen RP. Male factor subfertility: possible causes and the impact of nutritional factors. *Fertil Steril*; .2000.73: 435-442.
6. Tremellen K. Oxidative stress and male infertility-a clinical perspective. *Hum Reprod Update* 2008; 14: 243-258.
7. Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. *Reprod Biomed Online* 2007; 14: 727-733.

Comment [s16]: References number 9, 12, 13, 15, 19, 20, 26, 31, 33, 35 are missing in the references section.

There is need to revise the references as they only validates the scientific evidence of the study

8. Lafuente R, González-Comadrán M, Solà I, López G, Brassesco M, Carreras R, et al. Coenzyme Q10 and male infertility: a meta-analysis. *J Assis Reprod Gen* 2013; 30: 1147-1156.
11. Saleh RA, HCLD AA. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl* 2002; 23: 737-752.
16. Aitken R, Irvine D, Wu F. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Am J Obstet Gynecol* 1991; 164: 542-551.
17. Sukcharoen N, Keith J, Irvine DS, Aitken RJ. Predicting the fertilizing potential of human sperm suspensions in vitro: importance of sperm morphology and leukocyte contamination. *Fertil Steril* 1995; 63: 1293-1300.
25. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, et al. A systematic review of the effect of oral antioxidants on male infertility. *Reprod Biomed Online* 2010; 20: 711-723.
28. Moslemi MK, Tavanbakhsh S. Selenium-vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. *Int J Gen Med* 2011; 4: 99-104
29. Arduini A, Bonomini M, Savica V, Amato A, Zammit V. Carnitine in metabolic disease: potential for pharmacological intervention. *Pharm Ther* 2008;120: 149-156.
30. Radigue C, Es-Slami S, Soufir J. Relationship of carnitine transport across the epididymis to blood carnitine and androgens in rats. *Arch Androl* 1996; 37: 27-31.
32. Johansen L, Bøhmer T. Carnitine-binding related suppressed oxygen uptake by spermatozoa. *Arch Androl* 1978;1: 321-324.
34. Balercia G, Regoli F, Armeni T, Koverech A, Mantero F, Boscaro M. Placebo-controlled doubleblind randomized trial on the use of L-carnitine, Lacetylcarnitine, or combined Lcarnitine and Lacetylcarnitine in men with idiopathic asthenozoospermia. *Fertil Steril* 2005; 84: 662-671.
36. Garolla A, Maiorino M, Roverato A, Roveri A, Ursini F, Foresta C. Oral carnitine supplementation increases sperm motility in asthenozoospermic men with normal sperm phospholipid hydroperoxide glutathione peroxidase levels. *Fertil Steril* 2005; 83:355-361.

37. Wu Z, Lu X, Wang Y, Sun J, Tao J, Yin F, et al. [Short-term medication of L-carnitine before intracytoplasmic sperm injection for infertile men with oligoasthenozoospermia]. *Zhonghua Nan Ke Xue* 2012; 18: 253-256. (In Chinese)
38. Ernster L, Forsmark-Andree P. Ubiquinol: an endogenous antioxidant in aerobic organisms. *Clin Invest* 1993; 71: S60-S65.
39. Balercia G, Buldreghini E, Vignini A, Tiano L, Paggi F, Amoroso S, et al. Coenzyme Q 10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. *Fertil Steril* 2009; 91: 1785-1792.
40. Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Effects of the reduced form of coenzyme Q 10 (ubiquinol) on semen parameters in men with idiopathic infertility: a double-blind, placebo controlled, randomized study. *J Urol* 2012;188: 526-531.
44. Khan MS, Zaman S, Sajjad M, Shoaib M, Gilani G. Assessment of the level of trace element zinc in seminal plasma of males and evaluation of its role in male infertility. *Int J Appl Bas Med Res* 2011; 1: 93- 96
45. Hadwan MH, Almashhedy LA, Alsalman ARS. Oral zinc supplementation restore high molecular weight seminal zinc binding protein to normal value in Iraqi infertile men. *BMC Urol* 2012; 12: 32.
46. Hadwan MH, Almashhedy LA, Alsalman ARS. Study of the effects of oral zinc supplementation on peroxynitrite levels, arginase activity and NO synthase activity in seminal plasma of Iraqi asthenospermic patients. *Reprod Biol Endocrin* 2014; 12: 1
49. Ebisch I, Thomas C, Peters W, Braat D, Steegers-Theunissen R. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Hum Reprod Update* 2007; 13: 163-174.
50. Ebisch I, Pierik F, De Jong F, Thomas C, Steegers-Theunissen R. Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? *Int J Androl* 2006; 29: 339-345.
52. Flohe L. Selenium in mammalian spermiogenesis. *Biol Chem* 2007; 388: 987-995.

54. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003; 49: 83-94.
55. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *J Urol* 2009; 181: 741-751.
56. Galatioto GP, Gravina GL, Angelozzi G, Sacchetti A, Innominato PF, Pace G, et al. May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele? *World J Urol* 2008; 26: 97-102.
58. Gopinath P, Kalra B, Saxena A, Malik S, Kochhar K, Kalra S, et al. Fixed Dose Combination Therapy of Antioxidants in Treatment of Idiopathic Oligoasthenozoospermia: Results of a Randomized, Double-blind, Placebo-controlled Clinical Trial. *Int J Infertil Fetal Med* 2013; 4: 6-13.
60. Gvozdjáková A, Kucharská J, Dubravický J, Mojto V, Singh RB. Coenzyme Q10, α -Tocopherol, and Oxidative Stress Could Be Important Metabolic Biomarkers of Male Infertility. *Dis mark* 2015; 2015: ID 827941.
61. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed.; WHO Press: Geneva, Switzerland, 2010; ISBN 978-924-154-778-9
62. Al-Azemi, M.; Omu, A.; Fatinikun, T.; Mannazhath, N.; Abraham, S. Factors contributing to gender differences in serum retinol and α -tocopherol in infertile couples. *Reprod. Biomed. Online* 2009, 19, 583–590.
63. Colagar, A.H.; Marzony, E.T. Ascorbic Acid in Human Seminal Plasma: Determination and Its Relationship to Sperm Quality. *J. Clin. Biochem. Nutr.* 2009, 45, 144–149.
64. Akmal, M.; Qadri, J.Q.; Al-Waili, N.S.; Thangal, S.; Haq, A.; Saloom, K.Y. Improvement in Human Semen Quality after Oral Supplementation of Vitamin C. *J. Med. Food* 2006, 9, 440–442.
65. Matorras, R.; Pérez-Sanz, J.; Corcóstegui, B.; Pérez-Ruiz, I.; Malaina, I.; Quevedo, S.; Aspichueta, F.; Crisol, L.; Martínez-Indart, L.; Prieto, B.; et al. Effect of vitamin E

Comment [s17]: These references are not referred in the study

administered to men in infertile couples on sperm and assisted reproduction outcomes: A double-blind randomized study. *F&S Rep.* 2020, 1, 219–226.

66. Keskes-Ammar, L.; Feki-Chakroun, N.; Rebai, T.; Sahnoun, Z.; Ghozzi, H.; Hammami, S.; Zghal, K.; Fki, H.; Damak, J.; Bahloul, A. Sperm oxidative stress and the effect of an oral Vitamin E and selenium supplement on semen quality in infertile men. *Arch. Androl.* 2003, 49, 83–94.

67. Blomberg Jensen, M.; Nielsen, J.E.; Jorgensen, A.; Rajpert-De Meyts, E.; Kristensen, D.M.; Jorgensen, N.; Skakkebaek, N.E.; Juul, A.; Leffers, H. Vitamin D receptor and Vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Hum. Reprod.* 2010, 25, 1303–1311.

68. Blomberg Jensen, M.; Bjerrum, P.J.; Jessen, T.E.; Nielsen, J.E.; Joensen, U.N.; Olesen, I.A.; Petersen, J.H.; Juul, A.; Dissing, S.;

Jørgensen, N. Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa.

Hum. Reprod. 2011, 26, 1307–1317.

69. Hoek, J.; Steegers-Theunissen, R.P.M.; Willemsen, S.P.; Schoenmakers, S. Paternal Folate Status and Sperm Quality, Pregnancy Outcomes, and Epigenetics: A Systematic Review and Meta-Analysis. *Mol. Nutr. Food Res.* 2020, 64, 1900696.

70. Boonyarangkul, A.; Vinayanuvattikhun, N.; Chiamchanya, C.; Visutakul, P. Comparative Study of the Effects of Tamoxifen Citrate and Folate on Semen Quality of the Infertile Male with Semen Abnormality. *J. Med. Assoc. Thai* 2015, 98, 1057–1063.

71. Ahsan, U.; Kamran, Z.; Raza, I.; Ahmad, S.; Babar, W.; Riaz, M.H.; Iqbal, Z. Role of selenium in male reproduction—A review. *Anim. Reprod. Sci.* 2014, 146, 55–62.

72. Safarinejad, M.R.; Safarinejad, S. Efficacy of Selenium and/or N-Acetyl-Cysteine for Improving Semen Parameters in Infertile Men: A Double-Blind, Placebo Controlled, Randomized Study. *J. Urol.* 2009, 181, 741–751.

73. Zhao, J.; Dong, X.; Hu, X.; Long, Z.; Wang, L.; Liu, Q.; Sun, B.; Wang, Q.; Wu, Q.; Li, L. Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis. *Sci. Rep.* 2016, 6, 22386.

74. Colagar, A.H.; Marzony, E.T.; Chaichi, M.J. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nutr. Res.* 2009, 29, 82–88.

75. Ciftci, H.; Verit, A.; Savas, M.; Yeni, E.; Erel, O. Effects of N-acetylcysteine on Semen Parameters and Oxidative/Antioxidant Status. *Urology* 2009, 74, 73–76.

Comment [s18]: These references are not referred in the study

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