Tropical Biomedicine 24(2): 45–48 (2007)

Effects of vitamin E and soybean oil supplementation on sperm parameters in male Sprague-Dawley rats

Naseem, M.[1], Goh, Y.M.,[2] Hafandi, A.,[2] Amal, N.M.,[1] Kufli, C.N.[2] and Rajion, M.A.[2]
[1] Institute for Medical Research, Kuala Lumpur, Malaysia.
[2] Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia.
Email: naseem@imr.gov.my
Received 6 August 2007; received in revised form 14 September 2007; accepted 17 September 2007

Abstract. This study was performed to determine the effects of dietary vitamin E and soybean oil (a rich source of polyunsaturated fatty acids) on the sperm concentration and motility in male Sprague-Dawley rats. Rats with body weights between 250 – 300 gms were randomly allotted into five treatment groups with three animals each. The trial lasted 63 weeks inclusive of a one week acclimatization period. Rats were fed either CTRL (Base Diet + 5 % Soybean Oil + 3000 IU Vitamin E), BD Only (Base Diet Only), BDVitE (Base Diet + 3000 IU Vitamin E Only), BDSBO (Base Diet + Soybean Oil Only) or COMM (Imported Commercial Rat Pellets). At the end of the trial, the rats were euthanized and sperm concentration and motility were evaluated for both left and right testicles. It was found that although sperm motility had no significant difference across treatment groups, animals supplemented with adequate vitamin E and soybean oils had significantly higher concentration of sperms. It was also shown that vitamin E supplementation alone is more important than dietary fat supplementation in influencing sperm concentration in rats.

INTRODUCTION

Antioxidants played an important role in laboratory rat reproduction (Das *et al.*, 2006). Antioxidants including vitamin E have been shown to improve sperm motility and enhances semen quality and fertility of rats (Yousef *et al.*, 2003). In recent years, vitamin E supplements have been widely used in rats diets and the levels for enhancing production, and reproductive performance have been increased several fold (Chinoy & Sharma, 1998). Supplementation with Vitamin E has also been shown to increase total sperm output and sperm concentration (Sonmez *et al.*, 2007) in boars and poultry. Other studies have found improvements in sperm quantity and quality with supplemental Vitamins C and E (Cerolini *et al.*, 2006). Apart from the protective effects of Vitamin E on membrane integrity that safeguarded cellular functions (Yousef *et al.*, 2003), membrane fluidity is also an important determinant of normal cell functions. The membrane fluidity of sperm membrane is mainly dependent on lipid constitution and the degree of polyunsaturated fatty acids (PUFA) unsaturation. A higher degree of unsaturation of PUFA in the spermatozoa membrane was also shown to affect sperm quantity and concentration (Lenzi *et al.* 1996). However, the alteration of the cellular membrane fluidity could take significant amounts of time depending on treatment regime (Gurr *et al.*, 2002). In fact, most of the trials assessing the effects of vitamin E and fats in the literature involved shorter time frame as compared to the current trial. In view of the long term effects of both vitamin E and PUFA on sperm production and motility, therefore, the objective of this study was to investigate the effects of vitamins E and PUFA (from soybean oil) supplementation on sperm motility and sperm concentration in male Sprague Dawley rats.

MATERIALS AND METHODS

A total of 15 male Sprague-Dawley rats weighing between 250-300gms were obtained from the Laboratory Animal Resource Unit, Institute for Medical Research (IMR) Kuala Lumpur. The entire trial lasted 63 weeks inclusive of a 1-week acclimatization period. These rats were randomly assigned into five groups of three animals each. All the rats in this study were housed individually in plastic polycarbonate cages. These cages were placed on stainless steel racks and housed in naturally ventilated experimental rooms with temperature and humidity of about 30°C and 70% relative humidity respectively, with 12 hours light and dark cycles. These animals were fed with their respective diets for the trial at 20gms/day and water was available *ad libitum*. The feed as well as the water intake was recorded daily using an electronic weighing balance. Treatment diets were created by adding 5% (w/w) soybean oil and/or vitamin E supplement at 3000 IU (Blackmore's, Australia) to a Base Diet. Soybean oil was chosen as its PUFA is relatively stable to oxidation and therefore does not quench the vitamin E supplemented in the diet significantly (Gunstone, 1996). The Base Diet used in this study was deficient in both fats and vitamin E (<260 IU), and had the following macronutrient composition (as % dry weight) : protein (18.8 %), crude fibre (5.0%), crude fat (<3.0%), ash (8.0%) and the rest are made up of non-protein nitrogen. The treatment diet groups were CTRL (Base Diet + 5% Soybean Oil + 3000 IU vitamin E), BD Only (Base Diet Only), BDVitE (Base Diet + 3000 IU vitamin E only), BDSBO (Base Diet + Soybean Oil only) and COMM (imported commercial rat pellets – A balanced and complete diet for rats).

Upon termination of the trial, the rats were euthanized and sperm samples were obtained from both left and right vas deferens to analyze for percentage progressive motility (PPM). Sperm concentration was then determined by counting the cells on a hemacytometer as described by Seed *et al.* (1996). All procedures for the trial was approved by the Animal Care and Use Committee, Ministry of Health Malaysia, approval no. ACUC/KKM/02(3/2006)(6). Datasets were checked for conformance to normality, and then analysed using a one way analysis of variance (ANOVA) procedure for the effects of PUFA and vitamin E supplementation on sperm concentration and motility. Significantly different means were then elucidated using the Duncan's Multiple Range Test (DMRT). All test were conducted at 95 % confidence level using SPSS 14.0 software (SPSS Inc. Illionois).

RESULTS

In general, sperm motility was not significantly different across group. However, treatment groups supplemented with sufficient levels of vitamin E and fats, such as COMM and CTRL diets tend to have higher concentration of sperms. It was also noted that Vitamin E supplementation alone resulted in significantly higher sperm concentration as compared to fat (or soybean oil) supplementation alone. These findings further underscore the importance of vitamin E in the reproductive functions in rats. Groups that received little or no vitamin E supplementation were shown to have a lower sperm concentration as indicated by groups BD and BDSBO in Table 1.

DISCUSSION

This study revealed that sperm motility was not significantly different across the groups. This may be due to the large discrepancy in PPM values within treatment groups as indicated by their large standard error of means. The association of vitamin E deficiency with impaired male reproduction has been established for more than three decades, and traditionally it is called the 'anti-sterility' vitamin (Uzunhisarcikli *et al*, 2007). In the rats, vitamin E prevents lipid peroxidation of spermatozoa (Rao & Sharma, 2001). Uzunhisarcikli *et al.* (2007) reported that vitamin E ameliorates oxidative stress in spermatozoa helping to maintain optimum fertilizing ability. In addition, vitamin E is

Table 1. Sperm motility and concentration after 62 weeks of treatment (Mean ± Standard Error of Means)

Treatment groups	Sperm motility (Mean ± S.E.M. PPM)^{ns}	Sperm Count (Mean ± S.E.M. x 10^6 cells/mL)
CTRL	82.9 ± 10.5	35.5 ± 6.2^{abc}
BD	76.5 ± 14.7	28.6 ± 3.1^{ab}
BDVitE	78.1 ± 6.9	40.6 ± 4.8^{bc}
BDSBO	87.1 ± 6.8	25.9 ± 2.1^{a}
COMM	93.2 ± 3.2	43.2 ± 3.4^{c}

Means with different superscripts within column differed significantly at P<0.05; ^{ns} Not Significant

believed to be the primary components of the antioxidant system of the spermatozoa (Wang *et al.*, 2007), and is one of the major membrane protectants against reactive oxygen species (ROS) and lipid peroxidation (Yousef *et al.*, 2003). Therefore it was not surprising that vitamin E contributed significantly to the higher sperm concentration observed for groups COMM, BDVitE and CTRL. In contrast, fat supplementation, particularly those of the reactive n-6 fatty acids could be detrimental if it is done without antioxidant supplementation (Gunstone, 1996).

Vitamin E is an important determinant of sperm parameters in the rat. Supplementation of reactive fat types should be done in tandem with adequate amounts of antioxidants to ameliorate potential adverse effects due to lipid peroxidation.

Acknowledgements. We thank the Director of the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for the encouragement and permission to publish this paper. This work received funding from Institute for Medical Research and Faculty of Veterinary Medicine, University Putra Malaysia. We would also like to thank the staffs of Laboratory Animal Resource Unit, IMR and Veterinary Physiology Laboratory, FVM-UPM for their contributions in this study.

REFERENCES

Cerolini, S., Zaniboni, L., Maldjian, A. & Gliozzi, T. (2006). Effect of docosahexaenoic acid and a-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. *Theriogenology* **66**: 877–886.

Chinoy, N.J. & Sharma, A. (1998). Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. *Fluoride* **31**: 203-216.

Das, S., Maiti, R. & Ghosha, D. (2006). Management of fluoride induced testicular disorders by calcium and Vitamin-E co-administration in the albino rat. *Reproductive Toxicology* **22:** 606–612.

Gunstone, F. D. (1996). *Fatty acid and lipid chemistry.* Glasgow: Blackie Academic & Professional. pp 1-33.

Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). Lipid biochemistry – *An introduction*. 5th edition. Blackwell Science Ltd, UK, 320.pp.

Lenzi, A., Gandini, L., Maresca, V., Rago, R., Sgrò, P., Dondero, F. & Picardo, M. (2000). Fatty acid composition of spermatozoa and immature germ cell. *Molecular Human Reproduction* **6**: 226-231.

Rao, M.V. and Sharma, P.S.N. (2001). Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. *Reproductive Toxicology* **15**: 705–712.

Seed, J., Chapin, R., Oria, C., Dostal, R., Foote, M., Gary, H., Klinefelter, R., Perreault, S.D., Chrader, S., Eyler, A., Prando, R., Treinen, K., Veeramachaneni, N.R. & Ise, D. (1996). Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reproductive toxicology* **10:** 237-244.

Sonmez, M., Yuce, A. & Turk, G. (2007). The protective effects of melatonin and Vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated male rats. *Reproductive Toxicology* **23**: 226–231.

Uzunhisarcikli, M., Kalender , Y., Dirican, K., Kalender, S., Ogutcu, A. & Buyukkomurcu, F. (2007). Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. *Pesticide Biochemistry and Physiology* **87**: 115–122.

Wang, S., Wang, G., Barton, B.E., Murphy, T.F. & Huang, H.F.S. (2007). Beneficial effects of vitamin e in sperm functions in the rat after spinal cord injury. *Journal of Andrology* **28:** 2.

Yousef, M.I., Abdallah, G.A. & Kamel, K.I. (2003). Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Science* **76**: 99–111.