Reproductive parameters and oxidative stress status of male rats fed with low and high salt diet

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ABSTRACT

BACKGROUND: Deficiency of minerals and micronutrients has been reported to impair the process of spermatogenesis. Historically, salt has been used by women on their husbands to increase their libido, however, the role of salt diet on sperm parameters are yet to be ascertained. **AIM:** The present study was designed to determine the effect of low and high salt diet on sperm parameters, oxidative status and reproductive hormone levels of male rats. **MATERIALS AND METHODS:** A total of 18 rats were divided into three groups: Group I: (control) received 0.3% salt diet, Group II: low salt (received 0.14% salt diet) and Group III: high salt (received 8% salt diet). All animals were treated for 6 weeks; after which epididymal sperm parameters; oxidative stress markers (malondialdehyde, glutathione, catalase and superoxide dismutase) in the testes and epididymal tissues, as well as follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels were determined. **RESULTS:** The results showed decreased sperm count in the low salt diet rats while increased sperm count was observed in the high salt diet treated rats. Both low salt and high salt diet fed rats exhibited increased abnormal sperm cells and increased epididymal oxidative stress when compared with their respective control. FSH and testosterone levels were increased in the high salt fed rats while LH level was decreased when compared with the control values. **CONCLUSION:** This study suggests that both low and high salt diet play a negative role in the fertility of male rats.

KEY WORDS: Oxidative stress, salt, sperm count, sperm morphology, testosterone

INTRODUCTION

Minerals and micronutrients deficiency has been reported to impair reproductive functions.[1] The decrease in the consumption of salt, a major source of sodium and chloride in the body, has been a major medical advice in the management of hypertension and has become a public health policy in some countries.[2] However, many experts acknowledge that a gap exists in this practice and agree that additional research is required.[2-4] About 60-80 million couples suffer infertility each year[5] and male infertility accounts for 40% of infertility problems,[6] emphasizing the need for studies on male reproduction.

In the trial of antihypertensive interventions and management studies, altered sexual function, fatigue and sexual impotence has been reported in patients assigned to severe sodium restriction of 70 mmol/day.[7] This implies that low salt might impair the male reproductive function. Furthermore, the low salt dietary habit of the Yanomamo Indians of Northern Brazil has been suggested to be responsible for the recurrent pregnancy loss in their population.[8] Since, the male gamete contributes 50% of the genomic material to the embryo and contributes as well as to placental and embryonic development,[9-11] it is essential that the male factor parameters be explored. Hence this study determined the role of salt diet on sperm parameters such as sperm count, sperm motility and sperm morphology in rats. The study also explores the possible role of salt on the pituitary-testicular axis, which is crucial in the process of spermatogenesis.

Salt also known as sodium chloride is essential to life because it helps the body to maintain the balance of fluids in various compartments. It also serves as preservatives for food, used during cooking to provide

taste, for the treatment of diarrhea among many other uses. Over the years, human populations have lived at extremes of sodium intake and less is known on the effect of this varying extreme sodium consumption on reproductive functions. Research has shown that the Yanomamo Indians live on an average of 0.46 g/day (20 mmol/day), the Alaskan Eskimos ingest about 1.38 g/day (60 mmol/day) of sodium, as high as 8.85 g/day (385 mmol/day) of sodium ingestion in Korea and 13.8 g/day (600 mmol/day) of sodium is ingested in northern Japan.[12,13] At present, most of the adult populations around the world has a mean daily sodium intake greater than (>2.3 g) 100 mmol/day with a mean intake greater than (>4.6 g) 200 mmol/day in some Asian countries.[14]

Oxidative stress is defined as an imbalance between the cellular antioxidant defense systems and the production of reactive oxygen species (ROS).[15] Spermatozoa are highly susceptible to damage induced by ROS. This is due to the high content of polyunsaturated fatty acids within the plasma membranes and a low concentration of scavenging enzymes within the cytoplasm.[16-18] There is growing evidence that oxidative stress significantly impairs sperm function and plays a major role in the etiology of defective sperm function.[18-21] This may lead to the onset of male infertility through mechanisms involving the induction of peroxidative damage to the plasma membrane.[16,18,19,22]

One of the suggested mechanisms by which high salt diet induces hypertension is by the generation of ROS,[23] which when generated in excess of the normal capacity of the cellular or exogenous antioxidants will lead to oxidative stress, which has been reported to affect the process of reproduction.[20,24] Furthermore, low salt diet activates the renin angiotensin aldosterone system and excessive angiotensin generation has also been associated with oxidative stress. Increased ROS levels have been detected in 25-40% of the semen of infertile men,[22] as well as lower levels of antioxidants in the semen of infertile men.[20,25,26] Thus this study was designed to determine the effect of low and high salt diet on sperm parameters, pituitary-testicular hormone profile and oxidative status in testicular and epididymal tissues of male Sprague Dawley rats.

MATERIALS AND METHODS

Animals and treatment

A total of 18 Sprague Dawley male rats were used in this study aged 10 weeks with a weight range of 120-130 g. They were allowed to feed and drink water *ad libitum* and maintained in a well-ventilated animal house with 12 h light: 12 h darkness at room temperature. The experiment was conducted in accordance to the U.S. National Institute of Health on the care and use of laboratory animals. The male rats were divided into three groups of six rats each as described below:

- Group I: 0.3% NaCl (control normal diet[27])
- Group II: 0.14% NaCl (low salt diet)
- Group III: 8% NaCl (high salt diet).

Rat feed formulation was obtained from livestock feed content and subjected to proximate analysis at livestock feeds laboratory to ensure that rats received adequate balanced nutrients. Sodium ion content of the feed was then determined by atomic emission spectophotometry[28] and the sodium ion content was calculated as 0.12%. Salt was then added to the feed in a w/w ratio to make up for 0.3%, 0.14% and 8% for the control, low salt and high salt diet respectively. The animals were fed for 6 weeks, since 6 weeks high salt diet has been known to induce hypertension in rats,[29] after which the experimental parameters were determined. The testes and epididymal tissue samples were collected and processed for oxidative studies while serum was obtained for hormonal assay.

Sperm function analysis

After exposing the reproductive tract, the caudal epididymis was carefully isolated and minced with scissors in 1 ml of physiological saline to release the sperm. Each chamber of the hemocytometer was loaded with 10 ul of diluted sperm and allowed to stand or settle for 5 min. Counting was done under a light microscope at × 400 magnification. Sperm morphology was determined using the Eosin and Nigrosin stain. Briefly, 10 ul of eosin and Nigrosin was mixed with 40 ul of sperm suspension. The sperm suspension was incubated at 40°C for 5 min and then re-suspended with a micro-pipette. About 100 sperm cells/rats were morphologically examined under the microscope at ×400 magnification. Morphological abnormalities were classified as headless sperm, banana head, bent neck and bent tail.[30-32] Sperm motility was done by placing 10 ul of sperm suspension on the slide for microscopic evaluation at a magnification of ×400. About 100 sperm cells were examined and classified as either motile or immotile and expressed as a percentage.[33,34]

Biochemical analysis

The reduced glutathione (GSH) content of the testis and epididymis homogenate were determined using the method described by van Doorn *et al*.[35] The GSH determination method is based on the reaction of Ellman's reagent (5,5′-dithiobis (2-nitrobenzoic acid) DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate, which is yellow at 412 nm. The activity of the superoxide dismutase (SOD) enzyme in the testis homogenate was determined according to the method described by Sun and Zigman.[36] The reaction was carried out in 0.05 m sodium carbonate

buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005 N HCl. Catalase (CAT) activities was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein as described by Aebi.[37] Absorbance was recorded using Shimadzu recording spectrophotometer (ultraviolet 160) in all measurement. Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems. Moreover it is used as an indirect index of lipid peroxidation.[38] The method of Mihara and Uchiyama[39] was used in this study for the determination of MDA, which is based on its interaction with thiobarbituric acid to form a pink complex with absorption maximum at 535 nm.

Hormonal assay

An enzyme based immunoassay system was employed to determine testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in the serum samples collected. The protocol used for the analysis was as described by the kit producers (Immunometrics Limited, UK).

Statistical analysis

Data were presented as mean and standard error of the mean. When one-way analysis of variance showed significant differences among groups, Newman-Keuls *post-hoc* test was used to determine the specific pairs of groups that were statistically different. A level of $P < 0.05$ was considered to be statistically significant. Analysis was performed with the GraphPad Instat Version 3.05 (GraphPad Software, San Diego California, USA).

RESULTS

The sperm count of low salt fed rats were significantly reduced while that of high salt fed rats were significantly increased when compared with the control values at $P < 0.05$ [Table 1]. Both low salt and high salt diet fed rats had a significant increase in percentage abnormal sperm cells when compared with control values at $P < 0.05$, whereas no significant difference was observed in the sperm motility of both low and high salt fed rats when compared with control.

A significant increase in FSH and testosterone levels were observed in the high salt diet rats, however there was a significant decrease in LH when compared with control $P < 0.05$ [Table 2]. On the other hand, there was no significant difference in the FSH, LH and testosterone levels of low salt diet fed rats when compared with control rats.

Figure 1 shows the MDA levels in the testicular and epididymal tissues. Both low salt and high salt diet fed rats significantly increased the MDA level in the epididymis ($P < 0.05$). However, the increase in testicular MDA level was not significant in both low salt and high salt fed rats compared with control animals.

Table 1: Sperm characteristics of male rats in control and other experimental rats

Parameter	Control	Low salt	High salt
Sperm motility (%)	84±7.7	76±5.9	80±7.1
Percentage abnormal sperm cells	5.46±0.8	12.6±1.2*	9.9±1.3*
Sperm count (10^6/ml)	51.45±2.2	44.06±1.3*	59.6±2.3*

Values are expressed as mean±SEM, *n*=6. **P*<0.05 compared with control group.
SEM=Standard error of mean

Table 2: LH, FSH and testosterone levels of male rats in control and other experimental rats

Parameter	Control	Low salt	High salt
FSH (mIU/L)	7.2±0.21	7.25±0.22	10.9±0.57*
LH (mIU/L)	9.55±0.25	9.35±0.62	6.28±0.38*
Testosterone (nmol/L)	9.9±0.62	12.1±0.45	18.7±2.9*

Values are expressed as mean±SEM, *n*=6. **P*<0.05 compared with control group.
SEM=Standard error of mean; FSH=Follicle stimulating hormone; LH=Luteinizing hormone

Figure 2 shows the SOD activities in the testes and epididymis of control and experimental rats. Significant decreases in the activities of SOD in both the testicular and epididymal tissues were observed in both the low salt and the high salt fed rats when compared with the control ($P < 0.05$).

Figure 3 shows the GSH activities in the testes and epididymis of control and experimental rats. A significant decrease in the activity of GSH was observed in the epididymis of high salt fed rats when compared with the control ($P < 0.05$). No significant decrease in GSH activity was observed in the testes of high salt fed rats when compared with the control. In the low salt group the decrease in GSH activities were not significant in both the testes and epididymis when compared with the control.

Figure 4 shows the CAT activities in the testes and epididymis of control and experimental rats. Activities of CAT were significantly decreased ($P < 0.05$) in the testicular and epididymal tissues in both low and high salt fed rats when compared with the control.

DISCUSSION

Deficiency of minerals has been shown to impair reproductive functions. Sperm parameters such as count, motility and morphology are key indices of male fertility, as these are the prime markers in testicular spermatogenesis and epididymal maturation. Decreased sperm count, decreased motility and increased abnormal sperm cells are usually associated with decreased fertility rate.[40,41] The decrease in sperm count and increase abnormal sperm

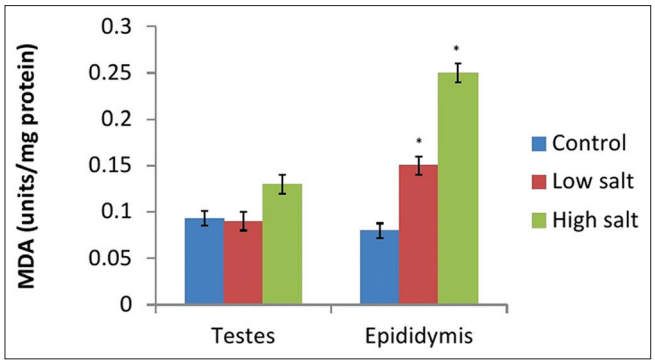

Figure 1: Effect of low salt and high salt diet on malondialdehyde level in the testes and epididymis of rats. Values represent mean ± standard error of mean (*n*=6), *P*<0.05 compared with control group

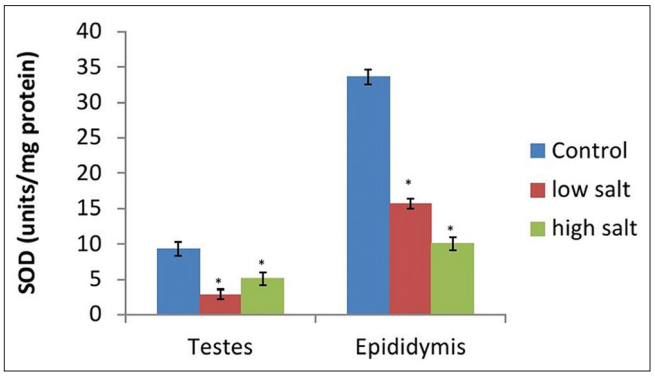

Figure 2: Effect of low salt and high salt diet on superoxide dismutase activity in the testes and epididymis of rats. Values represent mean ± standard error of mean (*n*=6), **P*<0.05 compared with control group

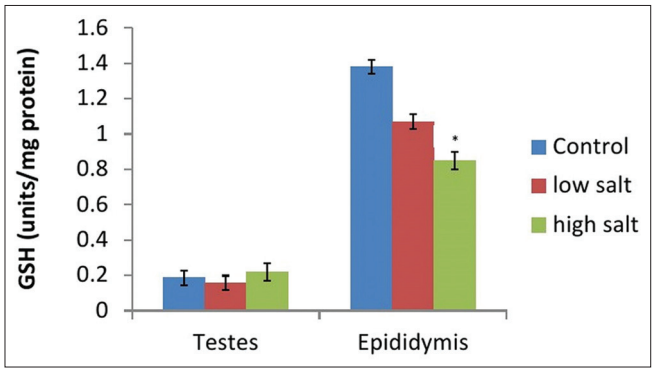

Figure 3: Effect of low salt and high salt diet on glutathione activity in the testes and epididymis of rats. Values represent mean ± standard error of mean (*n*=6), **P*<0.05 compared with control group

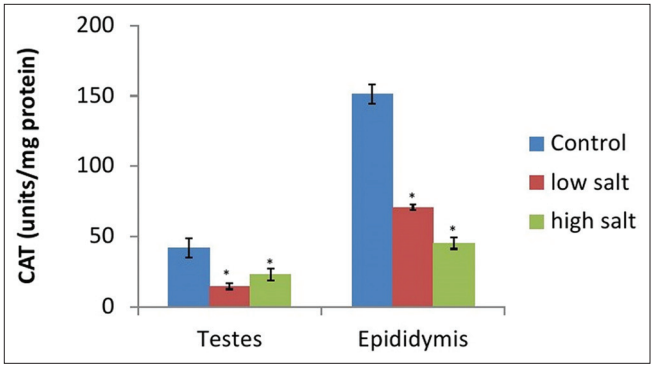

Figure 4: Effect of low salt and high salt diet on catalase activity in the testes and epididymis of rats. Values represent mean ± standard error of mean (*n*=6), **P*<0.05 compared with control group

cells in the low salt fed rats might indicate that low salt diet predisposes to decreased fertility. Testosterone and FSH are required to obtain full reproductive potential in males.[42] In this study, low salt did not alter FSH, LH and testosterone levels which imply that the decreased sperm count might not be via the pituitary-testicular axis. However, a decrease sperm count could ensue if the sertoli cells do not respond appropriately to testosterone actions, thereby leading to less production of growth factors that are required for the germ cell metabolism.[43,44] This might also be responsible for the increased number of abnormal sperm cells suggesting that the process of spermatogenesis might have been compromised by the administration of low salt diet.

Increased sperm count in the high salt diet rats might signify an increase in the fertility rate. In contrast to this, is the presence of a greater amount of abnormal sperm cells in the high salt diet fed rats. The increased sperm count in the high salt diet rats might be associated with the observed increase in the levels of serum FSH and testosterone, which are highly required for spermatogenesis. The decreased LH observed in this study in the high salt diet fed rats might be related to the negative feedback control action of testosterone. However, increased FSH could be a result of increased activin level, which is known to increase FSH binding and its biosynthesis and oppose the effect of inhibin on FSH.[45] Activin also participates in androgen synthesis by enhancing LH action in the testes. Therefore, it could be inferred that, high salt diet increased sperm count by stimulating the production of testosterone by a direct action on the leydig cells. Although sperm count was increased, the increased abnormal sperm cells present in the high salt diet animals might be due to oxidative stress damage.

It is well-known that normal sperm counts and other variables measured in routine semen analysis do not ensure good fertility rate. Other factors not determined in routine semen analysis, such as ROS, have been related to low fertility.[16,18,21,46] Though, ROS are known to be necessary for the normal functioning of sperm cells during fertilization, elevated levels of ROS is also a common cause of sperm malfunction.[47] Oxidative stress, as a result of the imbalance between ROS and antioxidants in the semen can lead to sperm damage, deformity and eventually male infertility.[18,48] The spermatozoa, in common with all cell types have developed an elaborate antioxidant defense system consisting of enzymes such as CAT, SOD and reduced GSH that scavenge

and suppress the formation of ROS.[49,50] Estimation of end products of lipid peroxidation such as MDA is an index of the extent of oxidative damage to cellular structures.[18]

In this study, increased MDA levels in the epididymis and decrease in antioxidant status in both low and high salt diet fed rats indicates the presence of oxidative stress. The epididymis which is the site for maturation and storage of sperm cells is of great importance to the index of male fertility. ROS appears to play a role in the apoptosis of spermatozoa, thus a decreased sperm count.[49,51] Therefore, overproduction of free radicals and hence oxidative stress may account at least in part for the decreased testicular function observed in low salt diet fed rats in this study. Furthermore, the excessive free radical generated by the epididymis of rats fed with low or high salt diet might be responsible for the increase abnormal sperm cells in both groups of rats.

CONCLUSION

The study reports low sperm count in the low salt diet rats, increased abnormal sperm cells in low salt and high salt diet fed rats, as well as oxidative stress in the epididymis of both low salt and high salt diet fed rats. These suggest that both high salt and low salt diet might play a negative role in the fertility of male rats.

REFERENCES

1. Smith OB, Akinbamijo OO. Micronutrients and reproduction in farm animals. Anim Reprod Sci 2000;60-61:549-60.
2. Alderman MH. Reducing dietary sodium: The case for caution. JAMA 2010;303:448-9.
3. Egan BM. Reproducibility of BP responses to changes in dietary salt: Compelling evidence for universal sodium restriction? Hypertension 2003;42:457-8.
4. Institute of Medicine. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, DC: National Academies Press; 2004.
5. World Health Organization. The World Health Report: Fighting Disease, Fostering Development. Geneva: WHO; 1996.
6. Fleming S, Green S, Hall J, Hunter A, Stoddart N, Fishel S. Analysis and alleviation of male infertility. Microsc Anal 1995;45:35-7.
7. Wassertheil-Smoller S, Blaufox MD, Oberman A, Davis BR, Swencionis C, Knerr MO, *et al*. Effect of antihypertensives on sexual function and quality of life: The TAIM Study. Ann Intern Med 1991;114:613-20.
8. Moinier BM, Drüeke TB. Aphrodite, sex and salt-From butterfly to man. Nephrol Dial Transplant 2008;23:2154-61.
9. Sutovsky P, Schatten G. Paternal contributions to the mammalian zygote: Fertilization after sperm-egg fusion. Int Rev Cytol 2000;195:1-65.
10. Miozzo M, Simoni G. The role of imprinted genes in fetal growth. Biol Neonate 2002;81:217-28.
11. Puscheck EE, Jeyendran RS. The impact of male factor on recurrent pregnancy loss. Curr Opin Obstet Gynecol 2007;19:222-8.
12. Institute of Medicine, Food and Nutrition Board. Panel on Dietary Reference Intakes for Electrolytes and Water. Dietary Reference Intakes: Water, Potassium, Chloride and Sulfate. Washington, DC: National Academics Press; 2004.
13. Froment A, Milon H, Gravier C. Relationship of sodium intake and arterial hypertension. Contribution of geographical epidemiology (author's transl). Rev Epidemiol Sante Publique 1979;27:437-54.
14. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: Implications for public health. Int J Epidemiol 2009;38:791-813.
15. Sies H. Biochemistry of oxidative stress. Angewandte Chemie 1986;25:1058-71.
16. Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: The balance of benefit and risk. Bioessays 1994;16:259-67.
17. de Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa: A balancing act between beneficial and detrimental effects. Hum Reprod 1995;10 Suppl 1:15-21.
18. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology 1996;48:835-50.
19. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: Physiology and pathology. Int J Androl 1997;20:61-9.
20. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ Jr, Agarwal A. The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 1999;14:2801-7.
21. Marti E, Mara L, Marti JI, Muiño-Blanco T, Cebrián-Pérez JA. Seasonal variations in antioxidant enzyme activity in ram seminal plasma. Theriogenology 2007;67:1446-54.
22. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 1992;57:409-16.
23. Dobrian AD, Schriver SD, Lynch T, Prewitt RL. Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity. Am J Physiol Renal Physiol 2003;285:F619-28.
24. Hung TH, Burton GJ. Hypoxia and reoxygenation: A possible mechanism for placental oxidative stress in preeclampsia. Taiwan J Obstet Gynecol 2006;45:189-200.
25. Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil Steril 1995;64:868-70.
26. Smith R, Vantman D, Ponce J, Escobar J, Lissi E. Total antioxidant capacity of human seminal plasma. Hum Reprod 1996;11:1655-60.
27. Martus W, Kim D, Garvin JL, Beierwaltes WH. Commercial rodent diets contain more sodium than rats need. Am J Physiol Renal Physiol 2005;288:F428-31.
28. Association of of official Analytical Chemists (AOAC) International. Infants formula and medical diets. Methods of Analysis. 17th edition, AOAC Maryland USA, 2000. Ch. 50. p. 15-8.
29. Adegunloye BJ, Sofola OA. Effect of dietary salt loading and high-calcium diet on vascular smooth muscle responses and endothelium function in rats. Clin Exp Pharmacol Physiol 1997;24:814-8.
30. Melissa R. Sperm morphology: in pathophysiology of the reproductive system, glossary of hypertexts for biomedical sciences. [Last updated 2004. Accessed 18 Dec 2013] from: http://www.vivo.colostate.edu./hbooks/pathphys/reprod/semeneval/morph.html.
31. Ali S, Adel S. Effect of curcumin on rat sperm morphology after the freeze-thawing process. Vet Res Forum 2013;4:185-9.
32. Lukaszewicz E, Jerysz A, Partyka A, Siudzińska A. Efficacy of evaluation of rooster sperm morphology using different staining methods. Res Vet Sci 2008;85:583-8.
33. Zemjanis R. Collection and evaluation of semen. In: Diagnostic and Therapeutic Techniques in Animal Reproduction. 3rd edn: William and Wilkins Company; 1977. p. 242.
34. Pant N, Srivastava SP. Testicular and spermatotoxic effects of quinalphos in rats. J Appl Toxicol 2003;23:271-4.
35. van Doorn R, Leijdekkers CM, Henderson PT. Synergistic effects of

phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. Toxicology 1978;11:225-33.
36. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem 1978;90:81-9.
37. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
38. Draper H, Hadley A. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-31
39. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271-8.
40. Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: Birth cohort study in 577 men in Scotland over 11 years. BMJ 1996;312:467-71.
41. Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 1995;332:281-5.
42. Walker WH, Cheng J. FSH and testosterone signaling in Sertoli cells. Reproduction 2005;130:15-28.
43. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr Rev 2004;25:747-806.
44. Skinner MK. Sertoli cell secreted regulatory factors. In: Skinner MK, Griswold MD, editors. Sertoli Cell Biology. San Diego: Elsevier Science; 2005. p. 107-20.
45. Xia Y, Schneyer AL. The biology of activin: Recent advances in structure, regulation and function. J Endocrinol 2009;202:1-12.
46. Huszar G, Vigue L. Correlation between the rate of lipid peroxidation and cellular maturity as measured by creatine kinase activity in human spermatozoa. J Androl 1994;15:71-7.
47. Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction 2007;134:31-40.
48. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: An update. Am J Reprod Immunol 2008;59:2-11.
49. Record IR, Dreosti IE, McInerney JK. Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. Br J Nutr 2001;85:459-64.
50. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 2004;44:275-95.
51. Mostafa T, Anis T, Imam H, El-Nashar AR, Osman IA. Seminal reactive oxygen species-antioxidant relationship in fertile males with and without varicocele. Andrologia 2009;41:125-9.

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