Red wine as a diluent supplement for counteract the deleterious effects of lipid peroxidation during liquid storage of aged roosters semen

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Abstract: This study is an attempt to enhance the resistance of roosters' semen to peroxidative damage during *in vitro* storage by supplementing the semen diluent of cockerels with the powerful antioxidant red wine. Cockerels at 65 weeks of age were randomly distributed into six treatment groups of 10 birds each. Semen was collected twice a week from all cockerels during the whole period of experiment (12 consecutive weeks). Fresh semen served as a control (T1); Treatments were semen extended with Al – Daraji 2 diluent (AD2D) alone (T2); extended semen supplemented with 4, 8, 12 or 16 ml of red wine / 100 ml of AD2D (T3, T4, T5 or T6, respectively). Semen samples were then stored at 4 – 6 ºC for 24 h, 48 h or 72 h. Results revealed that the addition of red wine into semen diluent (T3, T4, T5 and T6) resulted in significant ($p < 0.01$) improvement in mass activity, individual motility, viability and percentages of normal spermatozoa and intact acrosomes compared to T1 and T2 groups. Moreover, T6 (16 ml red wine / 100 ml of diluent) recorded the best results in relation to these five traits followed by the results of T5, T4 and T3, respectively. In conclusion, the supplementation of semen diluent composition with red wine significantly ameliorates quality of aged roosters semen that *in vitro* stored for up to 72 h. However, the beneficial effect of red wine found in the present study may be due to enhance sperm resistance to lipid peroxidation that naturally occurred during *in vitro* storage of avian semen.

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Key words: red wine, diluent, lipid peroxidation, liquid storage, semen, aged roosters.

1. Introduction

Antioxidants are compounds that protect biological system against the potentially harmful effects of processes or reactions that can cause excessive oxidations (Krinsky, 1992). By implication they may inhibit the pathogenesis and disorders of the many diseases and cases that involve oxidative reactions (Diplock et al., 1998). Antioxidants can be of endogenous or exogenous origin, and contribute to the complex and integrated biological antioxidant defense system, which normally protects Lefèvre et al. (2007) found that moderate consumption of red wine (equivalent to 2 glasses / day in humans) but not ethanol only, improves blood flow recovery by 32 % after bindlimb ischemia in hypercholesterolemic Apo – deficient mice. In ischemic tissues, red wine consumption reduces oxidative stress and increases capillary density by 46 %. Micallef et al. (2007) concluded that red wine consumption decreases oxidative stress and enhances total antioxidant capacity in the circulation. This decrease in oxidative stress and increase in total antioxidant capacity in circulation is important as the opposite set of circumstances has been implicated in the pathogenesis of cardiovascular disease. However, the results produced from this study suggest the potent antioxidant properties provided by red wine and potential protection from developing cardiovascular disease highlight the relationship between red wine consumption and health.

The phospholipids of spermatozoa are characterized by extremely high proportions of long – chain, highly polyunsaturated fatty acids. In birds fatty acids of the *n-6* series (mainly 20:4 *n-6* and 22:4 *n-6*) are predominate (Surai et al., 1997). There is considerable evidence that such fatty acids play an important role in sperm function since cases of impaired fertility have been associated with reduced amount of these polyunsaturates in spermatozoa (Kelso et al., 1996). However, the high degree of polyunsaturation typical of sperm lipids renders these gametes highly susceptible to lipid peroxidation, with the consequent risk of damage to cellular structures (Niki et al., 1993). In fact peroxidative damage to spermatozoa is believed to be a major cause of male subfertility (Sikka et al., 1995). Thus, the viability, storage efficiency and fertilizing ability of spermatozoa are highly dependent on the expression of an effective antioxidant capacity to these cells and in the surrounding seminal plasma. Therefore, the present study was conducted as an attempt to enhance the resistance against lipid peroxidation during *in vitro* storage of roosters' semen with red wine additive in a basic diluent.

2. Materials and Methods

Cockerels (White Leghorn strain, 65 weeks of age) were randomly allocated to six treatment groups with 10 birds in each group. They were raised in floor pens and fed a commercial diet containing 16.5 % crude protein and 2850 ME / kg. Semen collected from all roosters twice a week by abdominal massage (Lake and Stewart, 1978) during 12 consecutive weeks was used for the measurements. Two replicate samples of semen each consisting of polled material from five birds in each semen collection, were obtained for each treatment group. Treatment groups were as follows: T1 = fresh semen, T2 = semen diluted 1:1 with Al – Daraji 2 diluent (AD2D; Al – Daraji, 2004) and T3 – T6 = semen samples diluted 1:1 with AD2D supplemented with 4, 8, 12 or 16 ml of red wine (France and California Beringer Founder's Estate Cabernet Sauvignon, Beringer Blass Wine Estates, St. Helena, California) / 100 ml of diluent, respectively. However, the pH of diluents was adjusted to be 6.8 – 7.1 and the osmotic pressure 360 – 400 Mosm / kg H_2O. Semen samples were then stored at 4 – 6 ºC for 24 h, 48 h or 72 h. Aliquots of semen samples were removed at 0 h, 24 h, 48 h or 72 h after *in vitro* storage for further assessment of spermatozoa motility, viability and integrity. Spermatozoa motility (movement in a forward) was estimated on a percentage basis by using the microscopic method of Sexton (1976). Viability was evaluated by Fast green stain – Eosin B stain – glutamate extender (Al – Daraji et al., 2002). The proportion of morphologically normal spermatozoa was measured by using a Gentian violet – Eosin stain (Al – Daraji, 1998). Acrosomal abnormalities were determined according to the procedure reported by Al – Daraji (2001).

Changes in the motility, viability and morphology of spermatozoa after *in vitro* storage for certain periods (0, 24 h, 48 h or 72 h) were evaluated by analysis of variance. Differences between treatment groups' means were analyzed by Duncan's multiple range test, using the ANOVA procedure in Statistical Analysis System (SAS, 1996).

3. Results

Semen samples incubation for 0, 24 h, 48 h or 72 h in the presence of added red wine (T3, T4, T5 and T6) were associated with a significant ($P < 0.01$) increases in mass activity and individual motility compared to T1 and T2 groups (Figures 1 and 2). However, T6 (16 ml red wine / 100 ml) recorded the best results as regards these two traits followed by the results of T5, T4 and T3, respectively. Therefore, there were progressive improvements in mass activity and individual motility of semen samples stored for different storage periods with the increasing in the level of red wine incorporated into semen diluent.

Addition of appropriate concentrations of red wine (T3, T4, T5 and T6) to the semen diluent maintained viability, spermatozoa normality and acrosome integrity better than our control system (T1 and T2) irrespective of the storage period of semen samples (Figures 3, 4 and 5). Furthermore, T6 likewise recorded the highest values concerning live spermatozoa, normal spermatozoa and intact acrosomes followed by the values of T5, T4 and T3, respectively.

4. Discussion

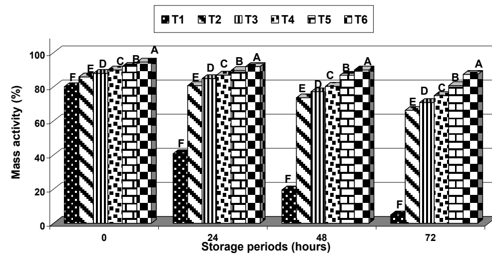
The results of present study clearly show that the addition of red wine to sperm extender provide the best protection against lipid peroxidation during liquid storage with regard to motility parameters, with the best results obtained for the 16 ml red wine / 100 ml of extender (T6). These results could be explained by that red wine considered as a very powerful antioxidant (Flesch et al., 1998). Hayek et al. (1997) reported that consumption of red polyphenols by mice significantly inhibited oxidative stress, atherogenesis and atherosclerotic lesion development. Azadzoi et al. (2005) concluded that the mechanism of oxidative stress injury is thought to involve lipid peroxidation, protein oxidation, DNA oxidation and decreased synthesis and bioavailability of endothelial and neuronal nitric oxide. However, oxidative injury is known to alter structure and function in many organs, including the heart, blood vessels, lung, kidney, brain, testis and arteriogenic erectile dysfunction. Da Luz and Coimbra (2004) concluded that an inverse association between moderate alcohol consumption, especially red wine, and cardiac mortality are extensive and highly suggestive, but still not definitive especially because they are based mostly on observation research. The Mediterranean diet, which included fish, red wine, fruits, and vegetables compared to the conventional prudent diet of the American Heart Association, significantly reduced the incidence of new coronary events by approximately 70%, over a period of 4 years (De Lorgeril et al., 1999). Rivero – Pèrez et al. (2007) showed that red wine exhibited important protective action on biomarkers of oxidative stress; they were much more active to inhibit lipid peroxidation than DNA oxidation. Su et al. (2006) noticed that glucose uptake by hepatocytes, adipocytes, and skeletal muscle and hepatic glycogen synthesis were all stimulated by resveratrol, red wine antioxidant, treatment. Because the stimulation of glucose uptake was not attenuated in the presence of an optimal amount of insulin in insulin – responsive cells, the antihyperglycemic effect of resveratrol appeared to act through

mechanism(s) different from that of insulin. Ruidavets et al. (2002) found a positive and significant association between red wine consumption and HDL and triglycerides and negative association with LDL in both sexes. Since increases in HDL are known to exert a protective effect against events due to coronary artery disease, this may also be a mechanism by which red wine protect against lipid peroxidation and atherosclerotic complications. The observation that red wine administration to healthy subjects increased plasma HDL cholesterol and apolipoprotein A – 1 plasma concentrations while white wine did not (Lavy et al., 1994), suggests that other components in red wine rather than its alcoholic content might play a role in cardiovascular prevention. Flavonoids and other red wine ingredients, which were recognized as strong antioxidants and oxygen free radical scavengers, enhanced in *in vitro* and in *ex vivo* experiments, the generation of nitric oxide, a platelet inhibitor and vasodilator (Andriambelson et al., 1997). Wollny et al. (1999) reported that remarkable difference ion phenolic components measured in red and white wine and the increased radical – trapping antioxidant activity in the plasma of rats given red wine strongly support the hypothesis that red wine antioxidant polyphenols may be implicated. Red wine contains a large number of compounds with antioxidant properties, including phenolic flavonoids, tannins, anthocyanins and natural antifungal compounds, such as trans – resveratrol (Rice – Evans et al., 1997). Wollney et al. (1999) also found that rats given red wine showed 4 fold increases in the total radical – trapping antioxidant parameter capacity as compared to controls or animals given white wine or ethyl alcohol. It was also found recently that moderate consumption of red wine protects rats from oxidation *in vivo* (Auger et al., 2002). Subsequently, the antioxidant properties of red wine phenolic compounds were shown to protect *in vitro* and *in vivo* LDL free radical – mediated oxidation (Nigdikar et al., 1998).

In this work, we have also shown that supplementation the roosters' extender wit red wine significantly decreases percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities. However, T6 (16 ml red wine / 100 ml of extender surpasses other treatments of red wine (T3, T4, T5 and T6) concerning these traits. These results are in accordance with studies of Al – Daraji (2002), Al – Daraji (2006 a), Al – Daraji (2006 b), Al – Daraji and Amen (2007) and Al – Daraji et al. (2007) who found that inclusion of certain synthetic or natural antioxidants into roosters' semen diluent resulted in significant amelioration in spermatozoa viability, morphology, motility, acrosome integrity and fertilizing ability when semen stored for 24 h at 4 ºC. It is speculated that the improvement in spermatozoa parameters are a result of antioxidant suppressing or limiting the damaging effects of lipid peroxidation *in vitro*. Enhancement of the antioxidant capacity of semen could present a major opportunity for improving male fertility. The beneficial consequences of an effective protection against lipid peroxidation are likely to result from two related mechanisms. (1) Defense against peroxidative damage is essential to maintain the structural integrity of the spermatozoa. (2) Minimization of lipid peroxidation will prevent and reduction in the concentrations of the functionally important C_{20-22} polyunsaturated fatty acids of the sperm phospholipids. Lipid peroxidation in biological membranes causes impairment of membrane functioning decreased fluidity, inactivation of membrane – bound receptors and enzymes, and increased non – specific permeability to ions (de Lamirande et al., 1997). Excessive generation of reactive oxygen species (ROS) in semen, mainly by neutrophils but also by abnormal spermatozoa, could be a cause for infertility. High concentrations of hydrogen peroxide induce lipid peroxidation and result in cell death. However, increased ROS production by spermatozoa is associated with a decreased mitochondrial membrane potential. The consequences of such oxidative stress include a loss of motility, viability and fertilizing potential, and the induction of DNA damage in the sperm nucleus. The loss of sperm function is due to the peroxidation of unsaturated fatty acids in the sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function (Sanocka and Kurpisz, 2004). However, the current methods of semen storage are only effective for short time (up to 12 h) and need to be improved. The use of low temperatures in combination with a buffered saline medium containing glycolytic substrates and intermediates of the citric acid cycle are not sufficient to ensure prolonged *in vitro* survival of avian spermatozoa (Thurston, 1995). Lipids are known to have a major impact on the structure and function of spermatozoa both *in vivo* and *in vitro*. Polyunsaturated fatty acids (PUFAs) of the membrane phospholipids may also be very sensitive to *in vitro* lipid peroxidation (Parks and Lynch, 1992). The importance of lipids in gamete biology is now well documented. They act *in vivo* in various physiological and metabolic processes, and are biological precursors of steroids, prostaglandins, and second messengers. In addition, they actively participate in the processes leading to gamete fusion. *In vitro* they are involved in mechanisms of cell resistance to cold shock and aerobic peroxidation and are believed to be metabolized actively (Douard et al., 2000).

On the other hand, Auger et al. (2000) found that red wine phenolic extract in the form of either reconstituted or alcohol – free wine had beneficial effects, i.e., hypochlesterplemic and to a lesser extent hypotriglyceridemic activities in hamsters because it has been reported that increased cardio – vascular risk reflected an increase in circulating cholesterol and triglycerides. Bitsch et al. (2004) reported that the content of total polyphenolics in plasma increased after intake of red grape juice and red wine with a c_{max} after about 30 minutes. Sharpe et al. (1995) noticed that red wine reduced LDL cholesterol, reduced LDL apo B and increased LDL cholesterol: apo B ratio, implying an increase in LDL size. Fuhrman et al. (1995) concluded that the antioxidant effect of dietary red wine on plasma lipid peroxidation was not secondary to changes in the plasma vitamin E or beta – carotene content but could be related to the elevation of polyphenol concentration in plasma and LDL.

In conclusion, developing a defense system against lipid peroxide damage is of practical importance to improving the extended liquid storage of avian semen. The present study demonstrated improved motility, survival, and membrane and acrosome integrity after cold storage of rooster spermatozoa with diluent supplemented with red wine as a strong antioxidant that scavenge ROS in the lipid membrane. Eventually, the fertilizing ability of spermatozoa is most important and future studies will evaluate the effect of this substance on this sperm function.



Figute1. Influence of diluent supplementation with red wine on mass activity of aged roosters semen.

T1= Fresh semen, T2= Semen extended with AD2D alone, T3 – T6= Semen extended with AD2D supplemented with 4 ml, 8ml, 12 ml or 16 ml red wine / 100 ml of diluent, respectively and AD2D = Al – Daraji 2 diluent.
Bars in each storage period with different superscripts differ significantly (p < 0.01)

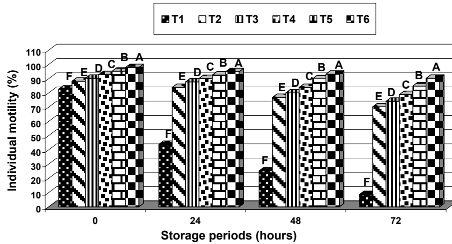


Figure2. Influence of diluent supplementation with red wine on individual motility of aged roosters semen.

T1= Fresh semen, T2= Semen extended with AD2D alone, T3 – T6= Semen extended with AD2D supplemented with 4 ml, 8ml, 12 ml or 16 ml red wine / 100 ml of diluent, respectively and AD2D = Al – Daraji 2 diluent.
Bars in each storage period with different superscripts differ significantly (p < 0.01)

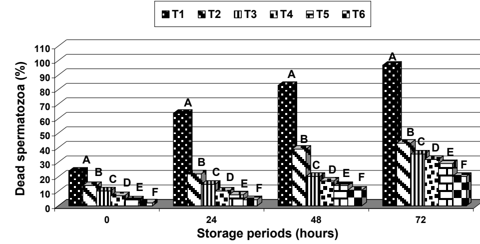


Figure3. Influence of diluent supplementation with red wine on percentage of dead spermatozoa of aged roosters semen.

T1= Fresh semen, T2= Semen extended with AD2D alone, T3 – T6= Semen extended with AD2D supplemented with 4 ml, 8ml, 12 ml or 16 ml red wine / 100 ml of diluent, respectively and AD2D = Al – Daraji 2 diluent.
Bars in each storage period with different superscripts differ significantly (p < 0.01)

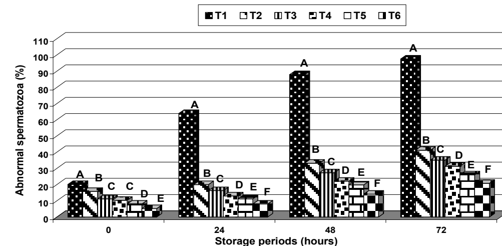


Figure4. Influence of dilluent supplementation with red wine on percentage of abnormal spermatozoa of aged roosters semen.

T1= Fresh semen, T2= Semen extended with AD2D alone, T3 – T6= Semen extended with AD2D supplemented with 4 ml, 8ml, 12 ml or 16 ml red wine / 100 ml of diluent, respectively and AD2D = Al – Daraji 2 diluent.
Bars in each storage period with different superscripts differ significantly (p < 0.01)

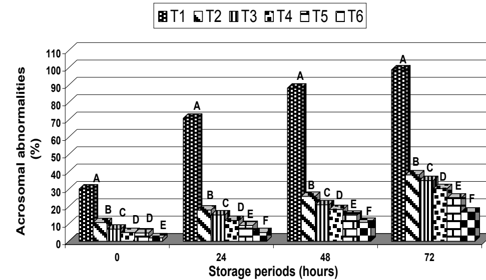


Figure 5. Influence of diluent supplementation with red wine on percentage of acrosomal abnormalities of aged roosters semen.

T1= Fresh semen, T2= Semen extended with AD2D alone, T3 – T6= Semen extended with AD2D supplemented with 4 ml, 8ml, 12 ml or 16 ml red wine / 100 ml of diluent, respectively and AD2D = Al – Daraji 2 diluent.
Bars in each storage period with different superscripts differ significantly (p < 0.01)

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References

1. Al – Daraji HJ (2006 a) Influence of supplementing diluent of semen with pomegranate juice on roosters semen quality during *in vitro* storage. *Iraqi Journal of Agricultural Sciences* **37 (4)**, : 149 - 158.
2. Al – Daraji HJ (2006 b) Effect of diluent supplementation with olive oil on susceptibility to lipid peroxidation during liquid storage of

aged roosters semen. *Iraqi Journal of Agricultural Sciences* **37 (2)** , 177 - 187.

3. Al – Daraji H J (2004) Diluent supplementation with vitamins A, C and E for improving fertilizing ability of indigenous roosters' semen. **Patent No. 3195**, issued from *Central Organization for Standardization Quality Control, Iraq.*

4. Al – Daraji HJ (2002) Effect of vitamins A, C or E on quality of fowl semen stored for 24 hours at 4ºC. *Iraqi Journal of Agriculture* **7(6)**, 170 – 181.

5. Al – Daraji HJ (2001) Effects of holding temperature and time on acrosomal abnormalities of fowl sperms. *Indian Journal of Animal Science* **71(1)**, 32 – 34.

6. Al – Daraji HJ (1998) Effect of ascorbic acid supplementation on physiological and productive traits of Fawbro broiler breeders' flocks reared under hot climate. *Ph. D. Dissertation, College of Agriculture, University of Baghdad.*

7. Al – Daraji HJ, Ameen MHM (2007) The addition of licorice extract to roosters' diluent for improve quality and fertilizing ability of spermatozoa of White Leghorn roosters. *Iraqi Poultry Sciences Journal.* **2 (2)**, 46 – 57.

8. Al – Daraji HJ, Razuki RH, Abdul – Hassan IA, Ahmed AS (2007) The relationship between the diluent provision of green tea infusion and lipid peroxidation during in vitro storage of roosters' semen. *Iraqi Journal of Agricultural Sciences* **38 (2)**, 141 – 147.

9. Al – Daraji HJ , Al – Tikriti BTO, Hassan KH, Al – Rawi AA (2002) New techniques for determination the avian spermatozoa abnormalities. *Journal of Biotechnological Research* **4(1)**, 47 – 64.

10. Andriambelson E, Kleschyov AL, Muller B, Beretz A, Stocklet J C, Andriantsitohaina R (1997) Nitric oxide production and endothelium – dependent vasorelaxation induced by wine polyphenols in rat aorta. *British Journal of Pharmacology* **120**, 1053 – 1058.

11. Auger C, Caporiccio B, Landrault N, Teissedre PL, Laurent C, Cros G, Besançon P, Rauanet J (2002) Red wine phenplic compounds reduced plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters (*Mesocricetus auratus*). *Journal of Nutrition* **132,** 1207 – 1213.

12. Azadzoi, KM, Schlman RN, Aviram M, Siroky MB (2005) Oxidative stress in arteriogenic erectile dysfunction: Prophylactic role of antioxidants. *Journal of Urology* **174(1)**, 386 – 393.

13. Balestriieri ML, Schiano, Felice F, Casamassimi A, Balestrieri A, Milone L, Servillo L, Napoli C (2008 a) Effect of low doses of red wine and pure resveratrol on circulating endothelial progenitor cells. *Journal of Biochemistry* **143 (2)**, 179 – 186.

14. Balestriieri ML, Fiorito C, Crimi E, Felice F, Crimi E, Felice F, Schiano C, Milone L, Casamassimi A, Giovane A, Grimaldi V, Del VG, Minucci PB, Servillo L, D'Armiento FB, Farzati B, Napoli C (2008 b) Effect of red wine antioxidants and minor polyphenolic constituents on endothelial progenitor cells after physical training in mice. *International Journal of Cardiology* **Feb. 4**, (Epub ahead of print).

15. Bitsch R, Netzel M, Frank T, Strass G, Bitsch I (2004) Bioavailability and biokinetics of anthocyanins from red grape juice and red wine. *Journal of Biomedicine and Biotechnology* 5, 293 – 298.

16. Burtns J, Gadner PT, McPhail DB, O'Neil J, Crawford S, Morecroft I (2000) Antioxidant activity, vasodilation capacity and phenolic content of red wines. *Journal of Agricultural and Food Chemistry* **48(2)**, 220 230.

17. da Luz PL, Coimbra SR (2004) Wine, alcohol and atherosclerosis: clinical evidences and mechanisms. *Brazilian Journal of Medical and Biological Research* **37(9)**, 1275 – 1295.

18. de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C (1997) Reactive oxygen species and sperm physiology. *Reviews of Reproduction* **2**, 48 – 54.

19. De Loreril M, Salen P, Martin JL, Monjand I, Delaye J, Manelle N (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. *Circulation* **99**, 779 – 785.

20. Diplock AT, Charleux JL, Crozier – Willi G, Kok FJ, Rice – Evans C, Roberfroid M (1998) Functional food science and defense against reactive oxidative species. *British Journal of Nutrition* **80 (Suppl. 1)**, S77 – S112.

21. Douard V, Hermier D, Blesbois E (2000) Changes in turkey semen lipids during liquid *in vitro* storage. *Biology of Reproduction* **63**, 1450 – 1456.

22. Duthie G, Crozier A (2000) Plant – derived phenolic antioxidants. *Current Opinion in Clinical Nutrition and Metabolic Care* **3**, 447 – 451.

23. Flesch M, Schwarz A, Bohm M (1998) Effects of red and white wine on endothelium –

dependent vasorelaxation of rat aorta and human coronary arteries. *American Journal of Physiology* **275**, H1183 – H1190.

24. Fuhrman B, Lavy A, Aviram M (1995) Consumption of red wine with meals reduces the susceptibility of human plasma and low – density lipoprotein to lipid peroxidation. *American Journal of Clinical Nutrition* **61**, 549 – 554.
25. Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, Elis A, Aviram M (1997) Reduced progression of atherosclerosis in apolipoprotein E – deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arteriosclerosis, thrombosis vascular biology* **17 (11)**, 2744 – 2752.
26. Kelso KA, Cerolini S, Noble RC, Sparks NHC, Speake BK (1996) Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. *Journal of Reproduction and Fertility* **196**: 201 – 206.
27. Krauss RM (2004) Hold the antioxidants and improve plasma lipids. *The Journal of Clinical Investigation* 113, 1253 – 1255.
28. Krinsky NI (1992) Mechanism of action of biological antioxidants. *Proceedings of the Society for Experimental Biology and Medicine* **200**, 248 – 254.
29. Lake PE, Stewart JM (1978) Atificial Insemination in Poultry. *Bulletin 213, Ministry of Agriculture, Fisheries and Food, London.*
30. Lavy A, Furhman B, Dankner G, Ben – Amotz A, Presser D, Aviram M (1994) Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation: Favorable effect of red wine on plasma high – density lipoprotein. Annals of Nutrition and Metabolism **38**, 287 – 294.
31. Lefèvre J, Michaud SE, Hadded P, Duuault S, Mènard C, Groleau J, Turgeon J, Rivard A (2007) Moderate consumption of red wine (cabernet sauvignon) improves ischemia – induced neovascularization in Apo E – deficient mice: Effect on endothelial progenitor cells and nitricoxide. *The Federation of American Societies for Experimental Biology (FASEB) Journal* **21 (4)**, 3845 – 3852.
32. Micallef M, Lexis L, Lewandowski PL (2007) Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans. *Nutrition Journal* **6**, 27 – 33.
33. Nigdikar SV, Williams NR, Griffin BA, Howard AN (1998) Consumption of red wine polyphenols reduces the susceptibility of low – density lipoproteins to oxidation *in vivo*. *American Journal Clinical Nutrition* **68**, 258 – 265.
34. Niki E, Noguchi EN, Gotoh N (1993) Polyunsaturated fatty acids in relation to sperm motility. *Journal of Andrology* **15**, 264 – 269.
35. Opie HL, Lecour S (2007) The red wine hypothesis: from concepts to protective signaling molecules. *European Heart Journal* **28 (14)**, 1863 – 1869.
36. Parks J, Lynch DV (1992) Lipid composition and thermotropic phase behavior of boar, stallion and rooster sperm membrane. *Cryobiology* **29**, 255 – 266.
37. Puddley IB, Croft KD. 1999. Alcohol, stroke and coronary heart disease. Are there antioxidants and pro – oxidants in alcoholic beverages that might influence the development of atherosclerotic cardiovascular disease? *Neuroepidemiology* **18**, 292 – 302.
38. Rice – Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**, 152 - 159.
39. Rivero – Pèrez MM, Muňiz P, González – Sanjosè (2007) Antioxidant profile of red wine evaluated by total antioxidant capacity, scavenger and biomarkers of oxidative stress methodologies. Journal of *Agricultural and Food Chemistry*, **Published on web 19 / 6 / 2007**, PAGE EST: 7.9.
40. Ruidavets JB, Ducimetiere PD Arveiler, Amouyel P, Bingham A, Wagner A, Cottel D, Perret B , Ferrieres J (2002) Types of alcoholic beverages and blood lipids in a French population. *Journal of Epidemiology and Community Health* **56**, 24 – 28.
41. Sanocka D, Kurpisz M (2004) Reactive oxygen species and sperm cells. *Reproductive Biology and Endocrinology* **2**, 12 – 30.
42. SAS (1996) SAS User's Guide: Statistics Version 6th edn. SAS Inc., Cary, NC.
43. Sexton TJ (1976) Studies on the dilution of turkey semen. *British Poultry Science* **17**, 179 – 186.
44. Sharpe PC, McGrath LT, McClean E, Young IS, Archbold GP (1995) Effect of red wine consumption on lipoprotein (a) and other risk factors for atherosclerosis. *QJM* **88 (2)**,101 – 108.
45. Sikka SC, Rajasekaran M, Hellstrom WJG (1995) Role of oxidative stress and antioxidants in male infertility. *Journal of Andrology* **16**, 464 – 468.
46. Su HC, Hung LM, Chen JK (2006) Resveratrol, a red wine antioxidant, possesses an insulin –

like effect in streptozotocin – induced diabetic rats. *American Journal of Physiology – Endocrinology and Metabolism* **209**, E1339 – E1346.

47. Surai PF, Kutz E, Wishart GJ,. Noble RC, Speake BK (1997) The relationship between the dietary provision of α – tocopherol and the concentration of this vitamin in the semen of chicken: effects on lipid composition and susceptibility to peroxidation. J*ournal of Reproduction and Fertility* **110**, 47 – 51.
48. Thurston R (1995) Storage of poultry semen above freezing for twenty – four to forty – eight hours. *In: Proceedings of First International Symposium on Artificial Insemination of Poultry*. Savoy, IL: Bakst and Wishart: 107 – 122.
49. Wollny T, Aiello L, DiTommaso D, Bellavia V, Rotilio D, Donati M B, deGetano G. , Iacoviello L (1999) Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. *British Journal of Pharmacology* **127**, 747 – 755.

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