Polyunsaturated Fatty Acids, Lipid Peroxidation and Antioxidant Protection in Avian Semen
- Review -

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ABSTRACT : Avian spermatozoa are characterised by high concentrations of polyunsaturated fatty acids (PUFAs), in particular docosatetraenoic (DTA, 22:4n-6) and arachidonic (AA, 20:4n-6) acids. As a result they are vulnerable to lipid peroxidation, which is considered to be an important factor of male infertility. Antioxidant systems are expressed in spermatozoa and seminal plasma and build three major levels of antioxidant defense. The first level is based on the activity of superoxide dismutase (SOD) which is, in conjunction with glutathione peroxidase (GSH-Px), catalase and metal-binding proteins, responsible for prevention of free radical formation. The second level of defence is responsible for prevention and restriction of chain reaction propagation and includes chain-breaking antioxidants such as vitamin E, ascorbic acid, glutathione and some others. The third level of antioxidant defence deals with damaged molecules, repairing or removing them from the cell and includes specific enzymes such as lipases, proteases, DNA repair enzymes etc. In the review, profiles of PUFAs and the two first lines of antioxidant defence in avian spermatozoa are characterised. Dietary manipulation of the breeder's diet (PUFA, vitamin E and selenium) as an effective means of modulating fatty acid composition and antioxidant system is also considered. Antioxidant properties of seminal plasma and efficiencies of inclusion of antioxidants into semen diluents are also characterised. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 7 : 1024-1050)*

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INTRODUCTION

Spermatozoa are highly specialized cells with a specific function, the fertilization of the egg. The high proportions of polyunsaturated fatty acids (PUFA) in the lipid fractions of spermatozoa reflect the need to maintain high membrane fluidity and the flexibility required for sperm motility and fusion with the oocyte. The high levels of PUFA render avian spermatozoa susceptible to lipid peroxidation and therefore these cells require adequate antioxidant capacities. Natural antioxidants (vitamin E, ascorbic acid and glutathione) associated with antioxidant enzymes (superoxide dismutase and glutathione peroxidase) combine to give avian semen an integrated antioxidant system capable of protecting against free radicals and toxic products of their metabolism. The delicate balance between free radical production and antioxidant defense is considered to be an important determinant of semen quality and in particular its fertilizing ability. The relationship between fatty acid profile and antioxidant protection in avian semen as well as the possibility of modulating these parameters by nutritional means has, until recently, received only limited attention and is considered in this review.

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POLYUNSATURAYED FATTY ACIDS IN AVIAN SPERMATOZOA

Lipids are major constituents of avian semen. They serve as structural compounds of the spermatozoan membranes, are precursors of different biologically active compounds (e.g. eicosanoids) and can be used for energy production.

Lipids in avian sperm include saturated (mainly 16:0 and 18:0), monounsaturated (mainly 18:1n-9; 18:1n-7 and 20:1n-9) and polyunsaturated (mainly 18:2n-6, 20:4n-6; 22:4n-6 and 22:6n-3) fatty acids (Surai et al., 1998; also see table 1). As can be seen from the data there are species-specific differences in the fatty acid profiles. For example, duck spermatozoa contained the highest proportion of PUFA whereas goose and turkey spermatozoa are characterized by much lower proportions of PUFA. As a result of those very high proportions of PUFA, duck and chicken spermatozoan lipids are characterized by the highest peroxidizability index compared to the other avian species.

Phospholipids are the major lipid fraction in avian spermatozoa, which, for example, form 66.4-70.7% of total sperm lipids in chicken (Cerolini et al., 1997a; Kelso et al., 1997a; Surai et al., 2000a) or 59.5% for the turkey (Cerolini et al., 1997a). It seems that the phospholipid proportion in the fowl spermatozoa increases with age reaching 72.1% of

Table 1. Polyunsaturated fatty acids in avian spermatozoa

	Chicken	Turkey	Guinea fowl	Duck	Goose
18:2n-6	2.6[a]	4.6[b]	3.8[abc]	3.8[abc]	5.7[bd]
20:2n-6	1.5[a]	4.0[ab]	3.3[ab]	1.6[a]	3.9[b]
20:3n-6	1.6	0.9	<0.5	1.0	0.5
20:3n-9	<0.5	2.7[a]	1.0[b]	<0.5	0.9[b]
20:4n-6	11.7[a]	9.4[b]	15.8[c]	18.9[d]	13.3[ac]
22:3n-9	3.8[a]	9.2[b]	4.3[a]	0.6[c]	1.4[c]
22:4n-6	27.9[a]	12.6[b]	19.4[c]	19.9[c]	17.5[cd]
22:5n-3	0.5	0.7	<0.5	0.6	<0.5
22:6n-3	2.1[a]	2.7[a]	1.7[a]	8.0[b]	2.8[a]
Total PUFA	52.1	46.8	49.3	54.4	46.0
Peroxidizability Index	215.0	184.0	195.1	231.3	185.6

[a,b,c] Means with different superscripts are significantly ($p<0.05$) different with respect to row. (Surai et al.,1998).

the total lipid fraction at 60 weeks of age (Kelso et al., 1996), but decreasing by 72 weeks of age to 51% (Kelso et al., 1997). In some cases the phospholipid proportion is reported to be 84.5% of the total lipids in fowl spermatozoa (Blesbois et al., 1997), but these authors did not detect the presence of triacylglycerol in the spermatozoa, which could comprise up to 4.2% (Cerolini et al., 1997a) or 7.3% (Kelso et al., 1997) depending on the age of cockerels.

The major PUFAs of the phospholipid fraction in fowl spermatozoa are shown in table 2. As revealed by these data, docosatetraenoic (DTA; 22:4n-6) and arachidonic acid (AA; 20:4n-6) are the major PUFAs in the phospholipid fraction of fowl spermatozoa. Complimentary information indicates that their proportions vary depending on male's age. DTA can be considered as the characteristic PUFA of avian spermatozoa while docosahexaenoic acid (DHA;22:6n-3) is the major representative of PUFA in mammalian spermatozoa (Poulos et al., 1973).

An additional feature of the PUFA composition in the phospholipid fraction of turkey spermatozoa is the comparatively high proportion of docosatrienoic acid (22:3n-9) (table 3). Phospholipids in duck spermatozoa contain the highest proportion of DHA and are also characterised by the presence of n-6 docosapentaenoic acid (DPA; 22:5n-6). The main phospholipids in chicken spermatozoa are phosphatidylethanolamine (PE) and phosphatidylcholine (PC). The total proportions of PE+PC range from 59-64% (Cerolini et al., 1997a; Kelso et al., 1996; Surai et al., 1997a), to 74% (Blesbois et al., 1997) of total phospholipids. Phosphatidylserine (PS) is also found in substantial quantities in fowl spermatozoa, comprising 11.3% (Blesbois, 1997), 21.3% (Surai et al., 1997a), 24% (Kelso et al., 1996), 19.8-28.9% (Cerolini et al., 1997a). The phospholipid profile of spermatozoa is dependent on the age of male birds. For example, in older fowl (72 wks) the PS proportion in spermatozoa comprised only 11.8% in comparison to 24.4% in young males (39 wks) (Kelso et al., 1997a).

In fowl spermatozoa the PE fraction was characterised by a higher degree of unsaturation compared to the PC fraction (table 4). The PS fraction was even more unsaturated than the PE fraction (Surai et al., 1997a). In turkey spermatozoa, the proportions of PE, PC, PS and sphingomyelin (Sph) were found to be 37.8; 38.9; 12.2 and 10.6% respectively (Cerolini et al., 1997a) and in duck spermatozoa the same phospholipid fractions comprised 28.0; 40.6; 11.2 and 9.1% respectively (Surai et al., 2000). As in the fowl, the PS fraction in duck spermatozoa was characterised by the highest proportion of the PUFAs which formed more than 77% of the total fatty acids.

Therefore the comparative data on the fatty acid composition of avian semen clearly indicate that PUFAs represent a major part (46.0-54.4%) of the total fatty acids. The spermatozoan lipids of 5 avian species investigated are characterized by a high peroxidizability index.

DHA is generally the most important spermatozoan PUFA in mammals, including man (Nissen and Kreysel, 1983), bull (Kelso et al., 1997b), monkey, (Lin et al., 1993), ram and boar (Poulos et al., 1973). However, in dog and rabbit spermatozoa, docosapentaenoic acid (DPA; 22:5n-3) is the main PUFA (Poulos et al., 1973). The importance of C_{22} polyunsaturates in relation to male fertility has been shown in humans where the amount of DHA in spermatozoa is positively correlated with sperm motility (Nissen and Kreysel. 1983; Zalata et al., 1998; Conquer et al., 1999) and with the percentage of morphologically normal sperm cells (Lenzi et al., 2000). Therefore the best morphological pattern corresponds to the highest DHA

Table 2. Main PUFA in phospholipid fraction of the chicken spermatozoa

	1	2	3	4	5	6	7	8	9	10	11
18:2n-6	2.3	2.5	2.3	2.8	2.1	3.6	2.7	3.4	3.7	6.0	2.6
20:4n-6	10.7	11.4	10.7	12.5	10.7	12.6	12.5	11.8	11.9	11.0	12.3
22:4n-6	18.7	21.7	18.2	30.5	16.6	22.3	22.8	22.9	21.7	17.8	22.1
22:6n-3	4.2	3.8	5.1	1.4	2.5	2.8	2.2	2.5	1.9	1.7	4.6

1. Surai et al., 1998b 45-50 w; 2. Kelso.et al., 1996 40w; 3. Kelso et al., 1996 58 w; 4. Cerolini et al., 1997a ISA, 23 w; 5. Surai et al., 1997a RIR, 24 w; 6. Cerolini et al., 1997a Cobb 38 w; 7. Kelso et al., 1997, 24 w; 8. Kelso et al., 1997, 39 w; 9. Kelso et al., 1997a 54 w; 10. Kelso et al., 1997a, 72 w; 11. Surai.et al,2000a, 60 weeks.

Table 3. Main PUFA in phospholipid fraction of turkey and duck semen

	Turkey	Duck
18:2n-6	1.4	1.8
20:4n-6	12.6	14.0
22:3n-9	7.1	-
22:4n-6	15.1	20.1
22:5n-6	-	11.2
22:6n-3	3.0	10.5

concentration in human semen (Lenzi et al., 2000a). In general, in mammalian spermatozoa long chain PUFAs containing 20-22 carbon atoms comprise more than 50% of total fatty acids in the phospholipid fraction and the level of DHA depends on the stage of sperm maturation (Ollero et al., 2000). In mammals, long chain PUFAs are actively produced during the maturation process of spermatozoa after their ejaculation from the testes (Lenzi et al., 2000a). In contrast to mammals, avian spermatozoa are less unsaturated and are characterised by the presence of DTA and AA which comprise more than 30% of total PUFA (Surai et al., 1998).

The biological reason for these species-specific differences in the PUFA profiles of avian spermatozoa is not clear at present. However, there is a growing body of evidence indicating that the fatty acid composition of sperm membranes, especially levels of PUFA, determine their biophysical characteristics such as fluidity and flexibility, both of which are necessary to fulfil specific functions, including motility and fertilizing capacity (Ladha, 1998). For example, increased PUFA concentrations in human spermatozoa were associated with increased sperm membrane fluidity (Comhaire et al., 2000). It is also necessary to appreciate that phospholipids containing DHA are not evenly distributed throughout the membranes of mammalian spermatozoa, being located almost entirely in the flagellum (Connor et al., 1998). Whether or not this is also the case for DTA in avian spermatozoa, has not yet been established. However, the very high proportion of long chain PUFA in the avian spermatozoa predisposes them to lipid peroxidation and it seems reasonable to suggest that antioxidant protection plays a crucial role in the maintenance of sperm membrane integrity and their fertilizing ability.

MECHANISMS AND CONSEQUENCES OF LIPID PEROXIDATION IN AVIAN SEMEN

Reactive free radicals (ex: hydroxyl radical) can initiate lipid peroxidation by a hydrogen abstraction reaction:

$$L\text{-}H + HO^* \longrightarrow L^* + H_2O \quad (1)$$

The propagation of lipid peroxidation occurs as follows:

$$L^* + O_2 \longrightarrow LOO^* \quad (2)$$

$$LOO^* + LH \longrightarrow LOOH + L^* \quad (3)$$

Since reaction 3 is the rate-limiting step of this chain-reaction, any substance that can reduce the concentration of peroxyl radicals (LOO*) will limit lipid peroxidation (Hogg, 1998). The main biological chain-breaking antioxidants are vitamin E, vitamin C and glutathione.

Toxicity of oxygen free radicals to the motility of human spermatozoa was observed more than 55 years ago (McLeod, 1943) and the toxic effect of H_2O_2 on chicken semen was shown by Wales et al. (1959). However, Jones and Mann published a milestone work in relation to lipid peroxidation in ram spermatozoa in 1973. These authors showed that ram spermatozoa produced, aerobically, an organic peroxide which can be determined quantitatively by the thiobarbituric acid reaction (Jones and Mann, 1973). They also proposed that lipid peroxidation may cause significant decline in respiratory rate and motility of spermatozoa. Lipid peroxidation irreversibly abolished the fructolytic and respiratory activity of spermatozoa. Moreover, the susceptibility of spermatozoa to peroxidation was increased in cells damaged prior to incubation (Jones and Mann, 1977a). Incubation of ram semen with peroxidized DHA caused the release of intracellular enzymes: lactate dehydrogenase (LDH), glutamic-oxalacetic transaminase (GOT), hyaluronidase, acid phosphatase and β-N-acetylglucosaminidase, into the surrounding medium, in a similar manner to enzymatic release observed after cold shock (Jones and Mann, 1977). Peroxidized PUFAs added to washed human sperm suspension immobilized the spermatozoa rapidly and permanently (Jones et al., 1978). Similarly, the toxicity of unsaturated fatty acids towards ram spermatozoa was shown to be directly related to their degree of peroxidation (Jones and Mann, 1977). Therefore lipid peroxidation can be considered as one of the biochemical causes of sperm senescence under storage conditions *in vitro* (Jones and

Table 4. PUFA in major phospholipid fractions of chicken semen

	PE 1	PE 2	PE 3	PE 4	PE 5	PC 1	PC 2	PC 3	PC 4	PC 5
18:2n-6	1.6	-	2.0	1.9	-	3.1	-	7.8	2.3	-
20:4n-6	23.6	28.9	22.2	15.6	14.0	8.4	9.7	7.8	6.1	9.6
22:4n-6	31.1	33.5	31.5	34.8	23.1	15.0	12.3	12.4	9.9	15.2
22:6n-3	4.3	Nd	7.9	6.1	5.8	1.4	nd	2.1	1.1	nd

1-Cerolini et al., 1997a, ISA, 23 w; 2- Kelso et al., 1996, Necked neck broiler, 25 w; 3- Cerolini et al., 1997a, Ross, 26 W; 4- Kelso et al., 1997, 40 w Ross; 5- Kelso et al., 1996, Necked neck broiler, 60 w.

Mann, 1976, 1977, 1977a) and loss of motility in human spermatozoa submitted to aerobic incubation appears to be the consequence of enhanced lipid peroxidation (Engel et al., 1999).

In mammalian spermatozoa, 22:6n-3 and 20:4n-6 were predominantly oxidized during sperm storage (Jones and Mann, 1976; Griveau et al., 1995). Thus, for human spermatozoa, peroxidation causes a dramatic decline of ethanolamine plasmalogen (from 10.1 to 4.3%) and PE (from 29.7 to 11,0%) while total amount of phospholipids decreased by about 30% (Jones et al., 1979). Using radio labelled fatty acids it was also demonstrated that PUFAs were predominantly incorporated into the plasmalogen fraction of phospholipid of human spermatozoa, while the peroxidation of PUFA from PE appeared to be very rapid (Alvarez and Storey, 1995). In this experiment, the level of arachidonic acid in phospholipids decreased from 3.9 to 2.6% and DHA from 47.5 to 27.2%. Peroxidation caused a loss of approximately 50% in the content of 20:4n-6 and 22:6n-3 fatty acids (Jones and Mann, 1976). Similarly, during peroxidation, phospholipids in human spermatozoan lost more than 65% of their DHA (Jones et al., 1978). MDA concentrations in asthenospermia and oligoasthenospermia were significantly higher than in normospermia and a negative correlation between MDA concentration and sperm motility was observed (Huang et al., 2000).

Research on lipid peroxidation of avian semen started in earnest with the publication by Fujihara and Howarth (1978) who demonstrated that during incubation at 41°C, fowl spermatozoa produced a thiobarbituric acid-reactive product while the susceptibility to peroxidation was enhanced by addition of ascorbate. Moreover, it was noted lipid peroxidation in spermatozoa was associated with a significant decrease of motility. In a more detailed investigation, Wishart (1984) found that the formation of high concentrations of MDA during a 5 h aerobic incubation of fowl semen was associated with a partial or complete loss of fertilising potential. It is interesting to note that the fertilising ability of semen samples producing low or negligible concentrations of MDA remained intact (Wishart, 1984). In this experiment semen samples incubated under anaerobic condition produced only negligible amounts of MDA. There was a considerable variability in individual males in relation to MDA production: 6 birds out of 48 studied produced lipid peroxide at a rate which was 70-fold greater than that of samples from other males (Wishart, 1984). This was hypothesised to be the result of compositional and functional differences in sperm membranes between individual males (Fujihara and Koga, 1992). Since all avian species are characterised by the presence of high concentrations of PUFAs in spermatozoa, lipid peroxidation is therefore a common process in sperm from any avian species. For example, incubating turkey semen in presence of Fe^{2+} induced lipid peroxidation and MDA accumulation (Surai, 1983; 1984). Moreover, an aerobic storage of turkey spermatozoa for several hours increased lipid peroxidation which was time- and temperature-dependent (Cecil and Bakst, 1993). These authors concluded that turkey spermatozoa underwent lipid peroxidation more readily than any other species previously studied. In a preliminary study with turkey semen, Donoghue and Donoghue (1997) showed that MDA concentrations were 10-fold higher in older toms (56 wk of age) than in younger toms (30 wk of age). This is in agreement with data of Kelso et al. (1996) indicating a 10-fold decrease in GSH-Px activity in spermatozoa collected from 60 wk versus 25 wk-old fowl males. It has also been observed that the antioxidant activity of bull seminal plasma decreased with aging (Fietta et al., 1982; Kelso et al., 1997b). Moreover, an accumulation of thiobarbituric acid reactive substances (TBARS) in duck semen as a result of lipid peroxidation has also been recently described (Surai et al., 2000). In general, MDA accumulation is positively associated with mid piece abnormalities in human spermatozoa (Aitken et al., 1993) or with a decrease in fertilising potential of fowl spermatozoa (Wishart, 1984; Cecil and Bakst, 1993). Both series of data are in agreement with an earlier suggestion that, due to inadequate superoxide dismutase (SOD) activity, lipid peroxidation may be a significant factor in poor semen quality and altered fertilizing potential in turkey males (Froman and Thurston, 1981).

Up to date, the molecular mechanisms of lipid peroxidation in avian semen have received scant attention. Moreover, information on the effect of storage of chicken sperm on the lipid composition is also limited. For example, no significant differences in total phospholipids or total neutral lipids were observed between control (recently ejaculated) and incubated (24 h; 41°C) fowl spermatozoa (Howarth, 1981). With an exception for sphingomyelin (Sph) content of which slightly decreased during incubation, the phospholipid composition remained stable. However, in another study the proportion of the PC and PS/PI fractions from seminal plasma appeared to increase over time (Ressequie and Hughes, 1984).

More recently it has been shown that during sperm storage lipid peroxidation is associated with a significant decrease in PUFA concentration. For example, incubation of chicken sperm for 12 h (20°C) was associated with a significant decrease in the proportion of 22:4n-6 in the phospholipid fraction (Surai et al., 1998b). The inclusion of Fe^{2+} in the incubation medium at 37°C further increased the rate of lipid peroxidation, decreasing the proportions of 20:4n-6, 22:4n-6, 22:5n-3 and 22:6n-3 in the phospholipid fraction present in spermatozoa. After 5 h of sperm incubation, the proportion of total C_{20-22} PUFA in the

spermatozoan phospholipid had decreased by 33% (Surai et al., 1998b). The loss of PUFA occurred simultaneously with the accumulation of TBARS in the semen. For example after incubating sperm for 30 minutes at 37°C in presence of Fe^{2+}, the accumulation of TBARS increased by approximately eight times in comparison to spontaneous peroxidation during sperm incubation without Fe^{2+}. Recently, the motility, viability, morphological integrity and lipid content have been measured in fowl semen stored for 48 h at 2 to 5°C and diluted 1:1 in Beltsville Poultry Semen Extender under aerobic conditions (Blesbois et al., 1999). The total lipid content, the proportion of total phospholipids, and the levels of PC, PE and Sph were significantly decreased and this was associated with a reduction in the proportion of motile, viable and morphologically normal cells.

In turkey spermatozoa incubated at 37°C in presence of exogenous Fe^{2+}, a significant decrease in PS (by 47%) and PE (by 35%), the two most unsaturated fractions of avian spermatozoa, was observed (Surai et al., 1998a; Maldjian et al., 1998). Storage of diluted turkey semen for 48 h at 4°C was also associated with a decrease in total phospholipid content and PC and to lesser extent in Sph, PS and PI (Douard et al., 2000). The significance of decreased concentration of these phospholipids requires further investigation. However PS appears to be an important phospholipid fraction in avian spermatozoa as it has the highest degree of unsaturation (Surai et al., 1997a; Surai et al., 2000), decreasing in chicken spermatozoa during ageing (Kelso et al., 1997, 1997a). Previous observations also demonstrated a significant positive correlation between PC concentration and the fertilizing ability of fowl sperm over the reproductive season (Cerolini et al., 1997).

In turkey semen stored for 48 h at 4°C, neither the total fatty acid profile nor the level of free cholesterol were affected but motility, viability and morphological integrity of spermatozoa significantly decreased (Douard et al., 2000). In contrast, as a result of the rapid lipid peroxidation occurring in turkey spermatozoa during incubation (1 h; 37°C, in presence of Fe^{2+}), there was a significant decrease in the levels of the main PUFAs: 22:4n-6, 20:4n-6, 22:6n-3 and 22:3n-9 (Surai et al., 1998a; Maldjian et al., 1999). Similarly, studies using liposomes have shown that 22:6n-3, 22:5n-3, 22:4n-6 and 20:4n-6 were very susceptible to peroxidation in an *in vitro* system (Sevanian and Hochstein, 1985). Therefore, the mechanisms by which ROS disrupt sperm function probably involves the peroxidation of PUFA in the sperm plasma membrane. For example, it has been shown that in human spermatozoa lipid peroxidation damages cell plasma membranes, leading to a loss of cytoplasmic components and hence causing progressive cell death. This process is considered to play an important role in the pathophysiology of human infertility (Aitken et al., 1993). Moreover, a negative correlation between the MDA production and sperm motility was observed in human semen (Huang et al., 2000).

However, the consequences of lipid peroxidation are not limited to membrane alterations but have other detrimental effects on cellular metabolism. For example, lipid peroxidation causes chromatin destabilisation associated with marked alterations in the DNA-protein complex. It also affects the functions of various enzymes, including cytochrome oxidase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase (Ferrandi et al., 1992; 1992a). Furthermore, ROS disrupt mitochondria functions, inhibit the synthesis of DNA, RNA and proteins (Comporti, 1989), increase DNA fragmentation (Lopes et al., 1998; Twigg et al., 1998), modify the cytoskeleton (Hindshaw et al., 1986), affect the axoneme (De Lamirande and Gagnon, 1992), and inhibit sperm-oocyte fusion (Aitken et al., 1993a). Therefore the detrimental effects of hydroxyl radicals on human sperm functions involve both lipid peroxidation and DNA modification (Chen et al., 1997). A significant positive correlation was observed between sperm MDA levels in humans and percentage of spermatozoa with a morphological stress pattern (Laudat et al., 1999). At the same time, inverse correlations between lipid peroxidation and the motility of the spermatozoa, their viability and their competence for sperm-oocyte fusion have been shown in humans (Gomez et al., 1998) suggesting that a significant proportion of infertile men carry high levels of TBARS and DNA damage in their ejaculated spermatozoa (Irvine et al., 2000). Therefore, the inverse relationship observed between antioxidant capacities and lipid peroxidation potential strongly suggests that impaired antioxidant defences play a significant role in infertile disorders (Smith et al., 1996).

Up to date most studies devoted to lipid peroxidation have been conducted in mammalian (mainly human) spermatozoa. Cryopreservation of sperm enhanced lipid peroxidation and this process was mediated at least in part by loss of SOD activity during the freezing procedures (Alvarez and Storey, 1992). A negative correlation between sperm motility and lipid peroxidation in cryopreserved semen samples has also been recorded (Bell et al., 1993). Furthermore, excessive generation of ROS and increased peroxidation in the membrane was hypothesized therefore as a biochemical basis for reduced activity of spermatozoa in cryopreserved semen (Askari et al., 1994). No doubt that lipid peroxidation in avian semen would have similar consequences. For example, it has been shown that H_2O_2 and organic hydroperoxides had toxic effects on sperm motility (Surai et al., 1998b). On a molar basis, H_2O_2 appeared to be more detrimental to sperm motility than cumene hydroperoxide as a result of higher permeability of plasma membranes to H_2O_2 than to organic hydroperoxides. Furthermore, avian spermatozoa appear more susceptible

than mammalian spermatozoa to H_2O_2 toxicity (Wales et al., 1959).

In order to better understand species-specific differences in lipid peroxidation in spermatozoa, it is necessary to take into account metabolic differences between the species studied, especially in terms of mitochondrial oxidative phosphorylation activity, the process during which superoxide radicals can be formed (Halliwell and Gutteridge, 1999). Finally, stress factors, capable of uncoupling oxidation and phosphorylation in mitochondria, can be considered as possible effectors stimulating electron leakage and superoxide radical formation. Another important consideration is the possible recycling of antioxidants (e.g. vitamin E) in spermatozoa. Because of very low activity or even absence of the hexose monophosphate shunt in avian spermatozoa (Sexton, 1974) the production of NADPH, the coenzyme for glutathione reductase, is limited. This means that recycling in the chain vitamin E - vitamin C - glutathione in the spermatozoa is limited as well. In such a situation, the primary defence preventing conversion of superoxide radical to more powerful radicals (for example, OH*) would be of major importance for sperm survival. An important feature of sperm metabolism is that spermatozoa cannot carry out extensive biosynthetic repair of damage (Hammerstedt, 1993). Therefore, any damaging alteration to membrane may irreversibly alter sperm functions. Finally, antioxidant protection appears to be absolutely vital to maintain fertilizing potential in avian sperm.

ANTIOXIDANT SYSTEMS OF AVIAN SEMEN

As discussed above spermatozoa are highly specialized cells consisting of various membrane structures. Their physical properties and functional integrity are essential to physiological functions, including motility and fertilizing ability. In this respect, the lipid composition of chicken semen is an important determinant of its quality (Cerolini et al., 1997). As has been shown above, avian spermatozoa are characterised by the presence within the phospholipids of high concentrations of C_{20} and C_{22} PUFAs, in particular, arachidonic (20:4) and docosatetraenoic (22:4) acids (Darin-Bennett et al., 1974; Howarth et al., 1977; Kelso et al., 1996, 1997, Cerolini et al., 1997a; Surai et al., 1997a, 1998, 2000, 2000a). The presence of high concentrations of PUFA within the lipids is an important risk factor for the development of lipid peroxidation within spermatozoa membranes and is generally considered to be a major cause of male subfertility (Wishart, 1984). Consequently, an efficient antioxidant system is required to protect sperm membranes against peroxidative damage (Surai, 1999, 2000).

The antioxidant protection in avian semen has, up to date, received limited attention. Recently, it has been suggested that the antioxidant system of spermatozoa includes three major levels of antioxidant defence (Surai, 1999, 2000) responsible for maintenance of biological functions of spermatozoa in various stressful conditions including sperm dilution, storage and deep freezing. Superoxide dismutase (SOD) in association with glutathione peroxidase (GSH-Px) and metal-binding proteins comprises the first level of antioxidant defense responsible for preventing the formation of free radicals (Surai, 2000). Natural antioxidants coupled with GSH-Px build the second level of antioxidant defence. They probably act to prevent and restrict the chain formation and propagation. The third level of defence is based on the enzymatic system responsible for repair or/and removal of damaged molecules in the cell. Fowl semen, generally, contains several natural antioxidants including vitamin E (Surai et al., 1997a, 1998b, 2000, 2000a), vitamin C and glutathione (Surai et al., 1998b) as well as antioxidant enzymes GSH-Px (Surai et al., 1998, 1998b) and SOD (Manella and Jones, 1980; Froman and Thurston, 1981; Surai et al., 1998, 1998b). Vitamin E was also found in turkey (Surai, 1981, 1989, Surai and Ionov, 1992), duck (Surai et al., 2000) and goose semen (Surai and Ionov, 1992a). Additionally, both, spermatozoa and seminal plasma contain antioxidant enzymes in pre-cited species (Surai et al., 1998).

Superoxide dismutase (SOD)

Since the superoxide radical is the main free radical produced in physiological conditions in the cell (Halliwell, 1994), superoxide dismutase (SOD; EC 1.15.1.1) is considered to be the main element of the first level of antioxidant defense in the cell (Surai, 1999, 2000). This enzyme dismutates the superoxide radical as follows:

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

There are three different forms of this enzyme in mammalian and avian species (Michalski, 1992). The main form is Mn-SOD that is located in mitochondria (Mates and Sanchez-Jimenez, 1999), a primary site of superoxide radical production (Halliwell and Gutteridge, 1999). Therefore the expression of Mn-SOD is considered to be essential for the survival of aerobic life and the development of cellular resistance to oxygen radical-mediated toxicity (Fridovich, 1995). A second form of this enzyme, Cu,Zn-SOD, is located in the cytosol (Harris, 1992). The third form of SOD, also called "extra-cellular SOD" is a secretory, Cu^{2+} and Zn^{2+} containing glycoprotein found in the interstitial spaces of tissues and extra-cellular fluids (Mates and Sanchez-Jimenez, 1999). The fourth form of the enzyme Fe-SOD was isolated from various bacteria but has, up to date, not been detected in animal tissues (Michalski, 1992).

Despite the importance of SOD in the protection of cells against lipid peroxidation, its activity in avian semen has received only limited attention. A comparison of SOD activity in sperm from various species including boar, rabbit, stallion, donkey, ram, bull, man and chicken indicated that donkey sperm had the highest and fowl the lowest SOD activity (Manella and Jones, 1980). Furthermore, turkey spermatozoa were found to contain even less SOD activity than fowl spermatozoa (Froman and Thurston, 1981).

Our recent data indicate that in seminal plasma of 5 avian species, KCN inhibited 100% of SOD activity, an observation reflecting the presence of only Cu, Zn-SOD (Surai et al.,1998). In the seminal plasma, the highest SOD activity was recorded in turkey and guinea fowl while the lowest activity was found in duck. Overall, avian species classified in accordance with decreasing SOD activity (expressed per mg seminal plasma protein) can be placed in the following order: guinea fowl>chicken>goose>duck> turkey. In contrast, the SOD activity in spermatozoa, from pre-cited species is classified in an opposite order to that observed in seminal plasma (goose>duck>chicken=guinea fowl>turkey, Surai et al., 1998). In sperm both forms of SOD are expressed with significant species-specific differences. For example in goose, Cu,Zn-SOD appears twice higher than Mn-SOD and an opposite distribution between different forms of SOD was recorded in guinea fowl where Mn-SOD was more than two-fold higher compared to Cu,Zn-SOD. In chicken, about 67% of total SOD activity was detected in spermatozoa as compared to 33% in seminal plasma (Surai et al., 1998b). The biological meaning and physiological consequences of such species-specific differences in SOD activity and distribution remain to be established.

Se and GSH-Px

That selenium is essential for male fertility became obvious in the early 1980s (Behne et al., 1982; Calvin et al., 1987; Wu et al., 1979; Hansen and Deguchi, 1996). This conclusion was based on the results of a range of different experiments with mammals. For example, in the case of a moderate deficiency, Se is preferentially retained in rat testes (Behne et al., 1982) while progressive selenium deficiency was associated with morphological alterations of spermatids and spermatozoa (Calvin et al., 1987). Subsequently, complete disappearance of mature germinal cells was observed (Behne et al., 1996). In selenium-deficient mice, the proportion of abnormal sperm ranged from 6.8% to 49.6% to compare to 4.0-15.0% in the control group. The most frequently occurring abnormalities in sperm shape were found in the sperm head. However, selenium deficiency also moderately increased abnormalities in other spermatozoa regions, including neck, mid-piece and tail (Watanabe and Endo, 1991).

It is noteworthy that mammalian semen contain the highest selenium concentration compared to other body tissues (Behne et al., 1986). Thus, in human semen, selenium was found mainly (more than 85%) in seminal plasma while sperm motility appeared maximal with semen Se levels ranging from 50 and 69 ng/ml (Bleau, 1984). Species-specific differences in Se levels in mammalian semen were also observed. For example, the seminal plasma level of Se was lowest in the human male and the stallion, higher in ram and boar, with the highest levels in the bull (Saaranen et al., 1989). In the human, a significant positive correlation between selenium concentration and the development of several reproductive organs including testes was observed (Oldereid et al., 1998). Selenocysteine is shown to be the main form of Se in rat sperm and selenocysteine and selenomethionine were found in ovine sperm (Alabi et al., 2000).

Selenium is a key component of a number of functional selenoproteins. Glutathione peroxidase (GSH-Px) is the best-characterized of the family of selenoproteins. In mammals it includes four members. The first member of this family, so called classical GSH-Px, was discovered in 1973 (Rotruck et al., 1973; Flohe et al., 1973). The second form of GSH-Px, the phospholipid hydroperoxide glutathione peroxidase (PH-GSH-Px), was described 9 years later (Ursini et al., 1982). The third member of this family, plasma glutathione peroxidase (pGSH-Px), was characterised in 1987 (Maddipati and Marnett, 1987; Takahashi et al., 1987). Recently, the fourth, selenoperoxidase: gastrointestinal glutathione peroxidase (GI-GSH-Px), has been described (Chu et al., 1993; for review Wingler and Brigelius-Flohe, 1999). These enzymes are characterized by different tissue-specificity and are coded by different genes (Ursini et al., 1997; Brigelius-Flohe, 1999). The major function of these peroxidases is considered to be removal and detoxification of hydrogen peroxide and lipid hydroperoxides (Ursini et al., 1997; Mates and Sanchez-Jimenez, 1999) according to the following reactions:

$$H_2O_2 + GSH\text{-}Px \longrightarrow H_2O$$

$$ROOH + GSH\text{-}Px \longrightarrow ROH$$

It is necessary to underline that H_2O_2, which is formed as a result of SOD activity is toxic and must be removed from the cell. In the case of low GSH-Px activity and in the presence of transition metals (Fe^{2+} and Cu^{+}) H_2O_2 can be converted into more powerful radicals including hydroxyl radical as follows:

$$H_2O_2 + Fe^{2+} \longrightarrow OH^* + OH^- + Fe^{3+}$$

The hydroxyl radical is considered as the most powerful biological radical capable of damaging major biological molecules including lipids, proteins, DNA and carbohydrates (Jaeschke, 1995). Also, hydroperoxides

(ROOH) produced as a result of the vitamin E reaction with the peroxyl radicals (ROO*) are toxic and, if not removed, impair membrane structure and function (Gutteridge and Halliwell, 1990). Lipid hydroperoxides are unstable and, in presence of transition metal ions, can decompose producing new free radicals and cytotoxic aldehydes (Diplock, 1994). Hydroperoxides therefore must be removed from the cell in the same way as H_2O_2, but CAT is not able to react with these radicals and only Se-dependent GSH-Px can deal with these radicals, converting them into non-reactive products (Brigelius-Flohe, 1999). Therefore, even in the presence of very high vitamin E concentrations animals including avian species need Se to maintain GSH-Px activity, an important step in prevention of lipid peroxidation and its toxicity. Since hydrogen peroxide is considered to be an intracellular messenger (Rhee, 1999) and since redox regulation can play a basic role in the activation of key transcription factors (Jackson et al., 1998; Dalton et al., 1999) it has been suggested that the regulation of delicate regional redox balance is one of the main function of glutathione peroxidases (Brigelius-Flohe, 1999).

Up to date, no data have been accessible to depict Se concentration in avian semen. However, GSH-Px has been found to be expressed in fowl seminal plasma and spermatozoa (Surai et al., 1998, a, c). Species-specific differences in activity and distribution of GSH-Px in avian semen have also been shown. For example total GSH-Px activity was significantly higher in turkey than in duck and goose seminal plasma (Surai et al., 1998). By contrast, the highest sperm GSH-Px activities were found in goose and duck and much lower GSH-Px activity was recorded in the guinea fowl, turkey and fowl. Recently, it has been shown that, despite high proportions of PUFAs and low levels of vitamin E, duck spermatozoa have the same susceptibility to lipid peroxidation as chicken spermatozoa (Surai et al., 2000), emphasizing an important role of an increased activity of Se-GSH-Px in duck semen. Studies performed with sperm issued from five avian species indicate that the Se-dependent form of GSH-Px comprised from 77.7% (chicken) up to 87.4% (guinea fowl) of total. In the fowl, approximately 60% of Se-GSH-Px was found in seminal plasma and 40% in spermatozoa (Surai et al., 1998, 1998b, c).

More generally, a GSH-Px activity has been found to be expressed in the semen from several other species including ram, dog, human, goat, bull (Li, 1975; Kantola et al., 1988; Kelso et al., 1997b). In bulls, GSH-Px is exclusively associated with seminal plasma (Brown et al., 1977; Smith et al., 1979). Furthermore, in seminal plasma a GSH-Px activity was low in man and ram, absent in boar and stallion but very high in bull (Saaranen, 1989). It was concluded that this selenium-dependent enzyme may be important in the protection of bovine spermatozoa against damages caused by oxygen radicals, while, in man, such a mechanism is not functional (Kantola et al., 1988). However, recent results of Bilodeau et al. (2000) indicate that bovine spermatozoa are poorly adapted to metabolize the toxic hydrogen peroxide, having very low levels of GSH-Px and no catalase activity. In some other species, for example in mouse spermatozoa, GSH-Px is considered to be the major protective system against oxidative damages (Ghyselinck et al., 1991) as a result of its action in decomposing lipid hydroperoxides - the key intermediates in spontaneous lipid peroxidation in mammalian sperm (Hansen and Deguchi, 1996). It has been shown that approximately two thirds of GSH-Px activity in bull semen was due to non-Se-GSH-Px (Slaweta et al., 1988). Moreover, MDA levels were found to be negatively correlated with Se-GSH-Px activity and it was suggested that Se-GSH-Px could play a role in protecting the acrosome membranes against disruption.

There is also a range of other selenoproteins identified in mammalian tissues but their functions are less characterized and their relation to avian semen production and quality remains unclear. Selenoproteins present in animal tissues include (Holben and Smith, 1999; Burk and Hill, 1999):

- thioredoxin reductase, an enzyme taking part in the reduction of protein disulphides and different physiological compounds, including dehydroascorbic acid;
- selenoprotein P, is present in plasma, playing roles in Se transport and serves a redox function in the extracellular space (Burk and Hill, 1994). In human plasma it accounts for approximately 40% of the total plasma selenium (Akesson et al., 1994);
- thyroid hormone deiodinases, enzymes are found in three different forms (Type I, Type II and Type III) which regulate the conversion of thyroxine to 3, 3, 5-triiodothyronine, the active thyroid hormone;
- selenoprotein W, was purified from rat muscle and was suggested to play a role in muscle metabolism;
- selenophosphate synthetase, an enzyme catalysing incorporation of the amino acid selenocysteine into selenoproteins;
- specific sperm mitochondrial capsule selenoprotein was identified in 1978 in rats (Calvin, 1978). Since then this protein has been extensively studied and it has been suggested that selenium is incorporated in the sperm mitochondria capsule and may thus affect behaviour and function of the spermatozoon. Recently this selenoprotein has been identified as PH-GSH-Px (Ursini et al., 1999) and it has been shown that PH-GSH-Px protein represents at least 50% of the capsule material embedding the helix of mitochondria (Ursini et al., 1999). A structural role of PH-GSH-Px in mature spermatozoa has therefore been suggested.

From the above studies it can be concluded that selenium is an essential trace element in vertebrates. A total of approximately 20 eucaryotic and over 15 procaryotic selenoproteins have, up to date been identified, partially characterised or cloned (Kohrl et al., 2000). Many of these proteins are associated with redox reactions in the cell. Tissue concentrations of selenoproteins are dependent on the dietary intake of selenium (Chen et al., 1990; Behne et al., 1991; Marchaluk et al., 1995; Persson-Moschos et al., 1998). Expression of individual selenoproteins is tissue-specific, depends on selenium availability, in some cases is regulated by hormones and if impaired contributes to various pathological conditions (Kohrle et al., 2000). Finally, selenoproteins discovered in mammalian cells should be considered as essential in the battery of body's antioxidant defenses, thyroid hormone function, immune system function, particularly the cellular immunity, formation of sperm and functionability of prostate (Badmaev et al., 1996).

Vitamin E

Vitamin E is generally considered as the main antioxidant of biological membranes (Niki, 1996). It reacts with free radicals as follows:

$$\text{Vitamin E} + \text{R-OO}^* \longrightarrow \text{Vitamin E}^* + \text{ROOH}$$

Vitamin E concentration in membranes is relatively low (< 1 mol per 1,000 mol of phospholipid; Packer, 1992). Nevertheless, due to its location inside the membrane at the water/lipid interface, vitamin E is able to effectively scavenge free radicals. Recently the idea that vitamin E may be recycled from its oxidised form has received significant attention. A series of experiments have demonstrated that the vitamin E recycling can be direct or indirect and mediated by either ascorbate (Chan et al., 1991; Chan, 1993), glutathione (Niki et al., 1982; Chan, 1993), cysteine (Motoyama et al., 1989), ubiquinols (Freisleben and Packer, 1993; Chan, 1993), lipoic acid (Packer, 1998), estrogens (Mukai et al., 1990) or carotenoids (Palozza and Krinsky, 1992; Bohm et al., 1997). Enzymatic regeneration of α-tocopherol has been also described (Maguire et al., 1989). The rate of reduction of phenoxyl radicals in the membrane decreased in the order of ascorbic acid>cysteine> glutathione (Niki, 1996). However, the rate of regeneration, or recycling, of the vitamin E radicals formed during their antioxidant action may affect both its efficiency in antioxidant action and its lifespan in biological systems. A greater recycling activity is associated with increased inhibition of lipid peroxidation (Packer, 1995). Whether all these regeneration reactions also occur *in vivo* await investigation. Due to incomplete regeneration (the efficiency of recycling is generally less than 100%) in biological systems, the antioxidants must be obtained from the diet (vitamin E and carotenoids) or synthesized in the tissues (ascorbic acid and glutathione).

Therefore the antioxidant protection in the cell depends on both, vitamin E concentration and location and effective recycling. Indeed, under normal physiological conditions the recycling is effective and even low vitamin E concentrations are able to maintain high antioxidant protection. An example of this action has been described in the brain of fowl embryos (Surai et al., 1996). In this tissue, products issued from lipid peroxidation are almost undetectable in physiological conditions irrespective on the very low vitamin E concentration.

In poultry, vitamin E was first detected in turkey semen (Surai, 1981). This study also demonstrated that most α-tocopherol could be detected in the cells and only a very low concentration of this vitamin was found in the seminal plasma. A similar distribution of vitamin E was shown for chicken semen with about 88% vitamin E to be located in the spermatozoa (Surai et al., 1998c). In general, depending on dietary supplementation vitamin E concentration in fowl semen may vary from 0.46 μg/ml (without vitamin E supplementation) up to 1.04-1.20 μg/ml (supplementation: 200 mg/kg) (Surai et al., 1997a; 1998c). When maize oil (5%) was included into fowl diet supplemented with 40 mg/kg vitamin E the concentration of α-tocopherol in the semen was 1.1 μg/ml (Surai et al., 2000a). Replacement of maize oil with tuna oil decreased vitamin E concentration in semen to 0.7 μg/ml. Moreover, the inclusion of arasco oil (high in 20:4n-6) in the diet (5%) and supplementation with 200 mg/kg increased vitamin E level in sperm up to 2.0 μg/ml (Surai et al., 2000a). However, the proportion of γ-tocopherol found in the sperm remained low (5-7% of total) compared to that observed in blood plasma (Surai et al., 1998b). It is also noticeable that vitamin E concentration in duck sperm only reached 48.1 ng/10^9 cells, a level approximately 3 times lower compared to that observed in the fowl (182.5 ng/10^9; Surai et al., 2000). In duck semen, most vitamin E was also located in spermatozoa. Information on vitamin E concentrations in sperm is quite limited, although some evidence exists that in mammalian spermatozoa the vitamin E levels is generally higher than that in avian species. For example, in boar semen, the α-tocopherol levels were found to be in the range of 365 - 497 ng/ml, which corresponds to about 1.600 μg/10^9 cells (Marin-Guzman et al., 1997). In sperm obtained from the caput epididymis of rats, vitamin E concentration was observed to be 3.56 μg/10^9 while in cauda epidymis spermatozoa its concentration was 0.94 μg/10^9 (Lenzi et al., 2000a). This was in agreement with data obtained in another study by Tramer et al. (1998). A wide range of α-tocopherol concentrations was detected in human

spermatozoa (10-245 ng/10^8 spermatozoa) and the percentage of motile spermatozoa was significantly related to sperm α-tocopherol content ($r=0.84$, $p<0.005$) (Therond et al., 1996). The level of α-tocopherol in semen was reduced from 5 μmol/l in men with normal semen parameters to 3 μmol/l as a result of oligospermia, azoospermia or asthenozoospermia (Omu et al., 1999). Similarly, vitamin E levels in seminal plasma of oligospermic and azoospermic men were significantly decreased as compared to the normospermic group (Bhardwaj et al., 2000). In rabbit semen, vitamin E concentration averaged 0.41 μmol/l and can be increased more than twice (up to 0.9 μmol/l) by increased dietary supplementation (Castellini et al., 2000). For comparison, in fowl semen vitamin E concentration comprises 1.64 μmol/l (Surai et al., 1998b). Vitamin E was found in the plasma membrane and acrosomal membrane of the rat epididymal spermatozoa and its localization in the tail may be associated with the mid-piece mitochondria (Lenzi et al., 2000a).

The associations between sperm vitamin E content and physiological and biochemical characteristics of spermatozoa have so far received only limited attention. Increased vitamin E concentration in turkey spermatozoa was associated with improved motility, viability and fertilizing ability after artificial insemination (Surai, 1983; 1991). In the fowl, increased vitamin E concentration in the chicken semen accompanied improved spermatozoa progressive motility (Cerolini, personal communication). In contrast increased vitamin E supplementation of the pheasant diet was associated with decreased fertility, however the duration of fertility was longer with vitamin E supplementation (Romboli et al., 2000). Positive effects of vitamin E were also observed in mammalian species. Thus, the α-tocopherol content in ram semen with 60-70% was higher than that observed in semen with only 30-40% motile spermatozoa (Kaludin et al., 1989). In spite of a range of tocopherols present in the feed, only α-tocopherol was detected in the ram seminal plasma and spermatozoa by paper chromatography-based method (Kaludin and Georgiev, 1984). A combination of increased levels of vitamin E and ascorbic acid in the rabbit diet was associated with significantly improved spermatozoa viability, the kinetics of spermatozoa movement and fertilizing ability (Castellini et al., 2000).

Ascorbic acid

Ascorbic acid is a water-soluble antioxidant generally present at high molar concentrations in both spermatozoa and seminal plasma for a wide range of animals. Thus in the fowl its concentration in semen averages 210.2±16.4 μM (Surai et al., 1998b). On a molar basis, this concentration was more than 2.5 times higher than glutathione and more than 100 fold higher than α-tocopherol. Ascorbic acid was almost equally distributed between the spermatozoa and seminal plasma (Surai et al., 1998b). These data were the background for a suggestion that ascorbic acid played an important role as a water-soluble antioxidant in avian seminal plasma.

In human seminal plasma, ascorbate contributes to antioxidant protection almost twice as much as urates (Lewis et al., 1997). Furthermore, thiol levels in human semen are about one third of ascorbate (Lewis et al., 1997). In seminal plasma of infertile men, ascorbic acid concentration was found to vary in a range as wide as 93-954 μmol/L (Gavella et al., 1997). Ascorbate concentration in seminal plasma of asthenozoospermic individuals exhibiting ROS activity was significantly reduced (Lewis et al., 1997). Ascorbic acid concentration in human seminal plasma was shown to be 612 μM and was negatively correlated with ROS production and positively correlated with percent morphologically normal spermatozoa (Thiele et al., 1995). Ascorbic acid also protects against endogenous oxidative DNA damage in human sperm (Fraga et al., 1991). However, the addition of ascorbate into sperm diluents (medium) did not modify the potential of sperm to fertilize ova (Tarin et al., 1994).

Glutathione

Glutathione (GSH) is the most abundant non-protein thiol in mammalian cells. It is considered to be an active antioxidant in biological systems providing cells with their reducing milieu (Meister, 1992). Cellular GSH plays a key role in many biological processes, including DNA and protein synthesis, cell growth and proliferation, regulation of apoptosis, immune regulation, transport of amino acids, xenobiotic metabolism and redox-sensitive signal transduction (Sen and Packer, 2000). Furthermore, GSH thiolic group can react directly with H_2O_2, superoxide anion, hydroxyl and alkoxyl radicals and hydroperoxides (Lenzi et al., 2000a; Meister and Anderson, 1983). Therefore, GSH acts as a free radical scavenger, particularly effective against the hydroxyl radical (Bains and Shaw, 1997), which remains intact in presence of the antioxidant enzymes. Usually, decreased GSH concentration in tissues is associated with increased lipid peroxidation (Thompson et al., 1992). Furthermore in tissues exposed to stress conditions, GSH prevents both protein thiol and vitamin E losses (Palamanda and Kehrer, 1993) while it also acts as an important key modulator of cell signalling (Elliott and Koliwad, 1997).

Glutathione concentration in fowl semen has been shown to be 83.7±9.12 μM (Surai et al., 1998b), equivalent to about 2.34 nmol/10^8 spermatozoa. In the rat, glutathione concentration was reported to be 3.5 nmol/10^8 spermatozoa

Table 5. PUFA in major phospholipid fractions of duck semen

	PE	PC	PS	Sp
18:2n-6	1.4	0.8	0.5	nd
20:4n-6	18.4	10.4	17.1	10.9
22:4n-6	19.5	5.5	17.8	4.8
22:5n-6	12.3	6.4	18.6	5.5
22:6n-3	10.3	6.7	23.6	8.2

(Surai et al., 2000)

Table 6. Lipid composition of seminal plasma, %

Fraction	ISA 23W	Cobb 26 w	Necked 25 W	Nacked 60 w	Turkey icholas 42 w
PL	46.2	46.9	34.2	28.4	40.5
FC	16.6	32.5	19.9	16.0	48.8
FFA	34.5	8.6	13.0	14.3	2.6
TG	3.0	2.3	22.0	20.1	2.1
CE	8.8	9.7	11.0	20.8	6.1
Mg lipid/ml	1.5	0.3	0.14	1.1	1.3

(Cerolini et al., 1997)

Table 7. Phospholipid composition of chicken seminal plasma, %

Phospholipid Fraction	25 weeks	60 weeks
PC	9.8	21.3
PE	52.6	34.6
PS	18.2	14.7
PI	Nd	10.6
Sph	19.4	7.5
CL	Nd	11.3

(Kelso et al., 1996)

Table 8. PUFA composition of the PL fraction of seminal plasma

PUFA	Egg type ISA	Broiler Breeder Cobb	Broiler Breeder Ross	Turkey Nicholas
18:2n-6	3.4	8.4	9.5	5.3
20:2n-6	<1	1.4	-	4.8
20:3m-6	1.3	2.5	-	1.2
20:4n-6	7.7	14.9	11.2	8.2
22:4n-6	14.4	10.7	8.3	10.5
20:3n-9	1.3	2.5	-	1.9
22:3n-9	<1	<1	-	4.7
22:5n-3	3.5	3.1	-	<1
22:6n-3	3.1	2.6	1.6	6.1

(Cerolini et al., 1997)

(Tramer et al., 1998). These data are quite similar to that observed in goat, rabbit, ram, dog, boar and human (Li, 1975). Recently, in human spermatozoa, glutathione concentrations have been reported to be: 3.49 nmol/10^8 spermatozoa in control subjects and less in patients with oligospermia, at 2.57 nmol/10^8 spermatozoa (Ochsendorf et al., 1998). Similar results were reported by Bhardwaj et al. (2000), showing that reduced glutathione was significantly lower in oligospermic and azoospermic group of human males. Glutathione concentration in bull spermatozoa was also similar: 3.1 nmol/10^8 spermatozoa (Slaveta and Laskowska-Klita, 1985).

Glutathione has been shown to play an important role in maintaining sperm motility and metabolism (Mann, 1981). The addition of glutathione into the incubation medium had a preserving effect on equine sperm motility maintained *in vitro* for 30 minutes under a free radical-generating conditions (Baumber et al., 2000). The introduction of GSH into the incubation medium caused a marked (by 57%) decrease of lipid peroxidation in boar spermatozoa (Brzezinska-Slebodzinska et al., 1995). Glutathione has also been shown to be protective against damage to isolated rat spermatids due to exposure to peroxidizing agents (Den Boer et al., 1990). Furthemore glutathione had a protective effect on rates of acrosome reaction and loss of motility over 24h in human spermatozoa prepared by centrifugation (Griveau and Le Lannou, 1994). In addition, GSH was effective in preventing the impairment of sperm motility observed in the presence of activated polymorphonuclear leuckocytes (Irvine, 1996). In contrast, the addition of glutathione to human sperm preparation medium had no significant effect on sperm progressive motility or DNA integrity (Donnelly et al., 2000).

LIPID AND FATTY ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF SEMINAL PLASMA

As can be seen from data presented in table 6, the lipid composition of seminal plasma is quite different from that of spermatozoa. Seminal plasma contains lower proportions of PL, and higher proportions of triacylglycerol and free fatty acids. There are also breeder-specific and age-related differences in the lipid composition of seminal plasma. The phospholipid composition of seminal plasma appears also different from that of spermatozoa. For example, the proportions of PC and Sph are lower and proportions of PE are higher. There are also dramatic age-related changes in the phospholipid composition of seminal plasma. Arachidonic acid (20:4n-6) and docosatetraenoic acid (22:4n-6) are major PUFAs present in both seminal plasma and spermatozoa. However, their proportions are somewhat lower than in spermatozoa.

After ejaculation, spermatozoa are protected from environmental stress by the combination of antioxidants in seminal plasma (Kobayashi et al., 1991). The observation that seminal plasma can protect spermatozoa against lipid peroxidation was first reported for mammalian semen. For

example, human seminal plasma effectively prevented, but did not reverse, the toxic effect of either endogenous or exogenous lipid peroxides on spermatozoa (Jones et al., 1978; Jones and Mann, 1979). In ram semen, lipid peroxidation can be inhibited by adding suspensions of dialyzed bull seminal plasma (Jones and Mann, 1977). However, dialyzed seminal plasma from ram, stallion and man had no protective effect on ram semen. Additionally, boar seminal plasma could inhibit lipid peroxidation in various *in vitro* systems (Smutna and Synek, 1979). The formation of lipid peroxides has been completely inhibited in bovine spermatozoa by the presence of seminal plasma in the incubation mixture (Dawra et al., 1983). A protective effect of rabbit seminal plasma against lipid peroxidation during sperm storage has also been described (Castellini et al., 2000a). The total antioxidant capacity of seminal plasma was significantly reduced in men with prostatitis and this was associated with increased generation of ROS (Pasqualotto et al., 2000). Furthermore, human seminal plasma protects the ultramorphological status of the sperm cells against deleterious effects of lipid peroxidation (Zabludovsky et al., 1999). Sperm oxidative damage induced by exogenous ROS, specifically DNA strand breaks and lipid peroxidation, was reduced by the presence of seminal plasma in the incubation medium (Potts et al., 2000). The protective effect of seminal plasma during human sperm cryopreservation (Ben et al., 1997) could also be related to its antioxidant potential.

In avian species, there is also evidence that seminal plasma can protect spermatozoa against peroxidation. For example, it has been shown that seminal plasma, calcium and citrate reduced peroxide production in the sperm by more than 3 times but bovine serum albumin and cholesterol had no protecting effect (Fujihara and Koga, 1984). In the same experiment fertilized eggs were obtained only from birds inseminated with sperm diluted with seminal plasma extended with phosphate buffer, Ca or citrate. A similar protective effect was observed with turkey semen (Cecil and Bakst, 1993). For example, turkey spermatozoa in diluent containing seminal plasma produced about 20% of the MDA compared to spermatozoa stored without seminal plasma. However, the washing out of chicken spermatozoa to eliminate seminal plasma increased MDA accumulation from 26.2 up to 41.3 μg/hour (Surai et al., 1998b).

To assess the antioxidant potential of seminal plasma several *in vitro* model systems have been used. The first system was based on chicken embryo brain homogenate. Since chicken brain is characterised by comparatively low antioxidant levels and contained very high proportions of PUFA (Surai et al., 1996), lipid peroxidation takes place during incubation of this homogenate and MDA is accumulated. Therefore, the introduction into this system of any compound possessing antioxidant activity would inhibit lipid peroxidation and decrease MDA production. In such a model system seminal plasma from ducks showed a 2-fold higher antioxidant activity compared to that of the chicken (Surai et al., 2000). The exposure of seminal plasma to elevated temperatures decreased its protective effect by 50%, which reflects the importance of thermolabile compounds, including the antioxidant enzymes SOD and GSH-Px, in the antioxidant activity of the plasma. A comparison of antioxidant activities of seminal plasma from boar, ram, human, turkey and chicken showed that human and turkey seminal plasma displayed the highest and that of chicken the lowest protective effect against peroxidation (Surai et al., 1998d).

The protective effects of seminal plasma against lipid peroxidation have been validated by using another *in vitro* system based on the production of free radicals during reaction between metmyoglobin and H_2O_2. Radicals produced were allowed to react with 2,2'-azinobis (3-ethyl-benzothiazoline 6-sulfonate) resulting in a green colour that was proportional to the extent of radical production. In this system, any substances possessing free radical-trapping activity are able to delay the reaction. The system is extremely sensitive and 5-10 μl of seminal plasma is sufficient to conduct an assay. It has been shown that the free radical-trapping activity of chicken seminal plasma is dose dependent (Surai et al., 1998b). In the same experiment, blood plasma free radical-trapping activity was only half that observed in seminal plasma. Additionally, incubation of seminal plasma for 24 hours at 20°C, or boiling for 10 minutes, did not reduce its free radical-trapping capacity (Surai et al., 1998b). Therefore plasma proteins are unlikely to play a major role in such a defense. The total antioxidant activity of fowl seminal plasma (Surai et al., 1998) was comparable with that observed in human seminal plasma (Gavella et al., 1996). In fact human seminal plasma possesses a high antioxidant buffering capacity that protects spermatozoa from oxidative stress (Rhemrev et al., 2000). Decreased seminal plasma antioxidant activity and increased reactive oxygen species production are considered to be responsible for idiopatic male infertility (Alkan et al., 1997). Among antioxidants, ascorbic acid, α-tocopherol and uric acid exert immediate, fast radical trapping, whereas hypotaurine and tyrosine give rise to the same slow radical trapping curve as seminal plasma (van Overveld et al., 2000). In general, human seminal plasma ascorbate, urates and thiols are considered to be major antioxidants (Lewis et al., 1997). Nevertheless compounds of the chicken seminal plasma responsible for this activity remain to be elucidated. Free radical-trapping activities of seminal plasmas were 13.2; 2.1; 2.1; 1.0 and 0.6 μM Trolox for turkey, goose, duck, guinea fowl and chicken respectively (Surai et al., 1998). It is interesting that

this activity is affected by lipid manipulation of the cockerel's diet (Cerolini et al., 1998).

The ranking of seminal plasma GSH-Px and SOD activities were almost the reverse of their ranking in spermatozoa, although dissimilar to total antioxidant activity of seminal plasma (Surai et al., 1998). This may reflect the presence of other natural antioxidants in seminal plasma, including ascorbate, glutathione, urate, albumin (Gavella et al., 1996) as well as some other low and high molecular weight factors (Kovalski et al.,1992).

Based on the very low level of vitamin E in avian seminal plasma (Surai et al., 1998b), it was suggested that vitamin E plays a minor role as a protective element of seminal plasma acting more probably as a stabilizer of sperm membranes as a result of its incorporation into sperm membrane structures (Surai, 1999). Indeed, the high proportions of 20:4n-6 and 22:4n-6 fatty acids in avian spermatozoa provide an ideal milieu for vitamin E incorporation into the membrane. However, precise mechanisms of vitamin E interactions with PUFAs in avian spermatozoa are not known and need further investigations. On the basis that it possesses higher concentrations of antioxidants than other biological fluids including blood serum (Lenzi et al., 2000a) seminal plasma is generally regarded as an excellent nutritive and protective medium for sperm cells.

In spite of the high antioxidant activity of seminal plasma, it is not able to totally prevent peroxidative damage to the spermatozoa during turkey and chicken semen storage (Donoghue and Donoghue, 1997; Surai et al., 1998b; Maldjian et al., 1998). In the past years, a number of attempts have been conducted to inhibit lipid peroxidation in spermatozoa by addition of antioxidants into the diluent. For example, inclusion of vitamin E into turkey diluent was shown to inhibit lipid peroxidation in the system containing Fe^{2+} (Surai, 1983, 1984). This work was expanded by Blesbois et al. (1993) who observed an improved fertilising ability of fowl semen stored for 24 h at 4°C in diluent supplemented with vitamin E. A direct supplementation of diluent with vitamin E was also shown to significantly increase resistance to lipid peroxidation and improve semen viability during storage at 4°C for 24-72 h (Surai et al., 1997). The addition of vitamin E (1,000 ppm) into the diluent was effective in preserving turkey sperm viability up to 48 h and motility up to almost 6 h of incubation at room temperature (Maldjian et al., 1998).

Introducing catechin and green tea extracts (100 ppm) into diluent significantly increased the proportion of intact spermatozoa after 48 h of incubation but had no effect on motility index. PL fatty acid content was dramatically affected during induced peroxidation as a general decrease in the proportion of all long chain PUFAs was observed. The protective effect of vitamin E was associated with prevention of PUFA oxidation. For example, the introduction of α-tocopherol into the incubation medium for turkey semen completely prevented loss of 22:6n-3, 22:5n-3 and 22:4n-6 due to peroxidation. Therefore, PL fatty acid profiles of spermatozoa incubated in presence of vitamin E (200 ppm) were similar to the control before incubation. However, it was not possible to achieve 100% protection from peroxidation, since the level of 20:4n-6 was still lower ($p<0.01$) compared to non-incubated spermatozoa (Surai et al., 1998a; Maldjian et al., 1998). In addition, PE and PS were the PL fractions that were the most affected during induced peroxidation. Again, the addition of vitamin E into the incubation medium prevented the changes in the proportion of turkey sperm PE and PS due to lipid peroxidation. Similarly, the addition of antioxidants (vitamin E, BHT, and "Tempo") to extended turkey semen improved sperm survival, membrane integrity, and reduced motility decline after a 48 h storage period (Donoghue and Donoghue, 1997). In the same experiment, ascorbic acid was not shown to have any effect on sperm storability.

α-Tocopherol significantly reduced the susceptibility of boar spermatozoa to lipid peroxidation and its inclusion into a diluent improved the viability of stored cells (Cerolini et al., 2000). Furthermore, the inclusion of α-tocopherol into the diluent was effective in preventing the decrease of 22:6n-3 observed in boar sperm phospholipid during *in vitro* storage (Cerolini et al., 2000). The addition of vitamin E to pre-freeze media for ram semen considerably increased sperm quality (Ollero et al., 1998). Vitamin E has also been successfully used to prevent oxidative damage to human spermatozoa during sperm preparation procedures (Aitken and Clarkson, 1988) while the introduction of α-tocopherol into the incubation medium protected human spermatozoa from the loss of motility as a result of peroxidation (Aitken et al., 1989). The inclusion of α-tocopherol in the medium during Percoll preparation of human sperm also protected DNA from damage induced by 30 Gy X-irradiation (Hughes et al., 1998). The incubated samples of ram semen with the addition of selenium and methionine contained higher amounts of α-tocopherol as against the control samples (Kaludin and Dimitrova, 1986). It is interesting that, in bovine sperm suspensions, incubated with vitamin E SOD activity was higher than in the control samples (Beconi et al., 1991).

Therefore inclusion of vitamin E into the diluent is proven to be an effective means of prevention of lipid peroxidation during sperm storage. However, optimal vitamin E doses need further investigation since the inclusion of vitamin E in chemically-defined ram semen diluent inhibited spermatozoal motility (Upreti et al., 1997). When vitamin E and vitamin E + vitamin C were added to the capacitation medium, a significant decrease in the percentage of capacitated spermatozoa was observed

(Oflaherty et al., 1997). In another study, high vitamin E concentrations in the medium also prevented sperm capacitation (Dalvit et al., 1998).

The addition of glutathione or its combination with hypotaurine, to human sperm preparation medium had no significant effect on sperm progressive motility or baseline DNA integrity (Donnelly et al., 2000). Ascorbic acid in concentrations below 1,000 μM protected human spermatozoa from free radical damage, thus improving their motility and viability and decreasing MDA generation. However, higher ascorbic acid concentrations were not protective, as abrupt decrease of sperm motility and viability and stimulated lipid peroxidation were observed (Verma and Kanwar, 1998). Sperm DNA can be protected from X-radiation damage by ascorbic acid (600 μM), α-tocopherol (30 and 60 μM) and urate (400 μM) (Hughes et al., 1998). Ascorbic acid (0.45 and 0.9 g/L) significantly increased the percentage of membrane-intact stallion spermatozoa at 24, 48 and 72 h at 5°C, but decreased the percentage of progressively motile spermatozoa at a concentration of 0.9 g/l in glycine extender (Aurich et al., 1997). However, the antioxidant protective effect can significantly vary since it has also been shown that supplementation of preparation media with ascorbate and α-tocopherol, either singly or in combination, was not beneficial to human sperm decreasing progressive motility, linearity and average path velocity (Donnelly et al., 1999) and inducing DNA damage (Donnelly et al., 1999a). Inclusion of ascorbic acid in the diluent for ram spermatozoa was not effective in improving post-thaw motility and in concentrations higher than 50 mM significantly reduced all motility characteristics (Sanchez-Partida et al., 1997). Similarly, in a randomized, placebo-controlled, double-blind study high oral doses of vitamins C and E for 56 days were not able to improve semen parameters in infertile men (Rolf et al., 1999). Nevertheless, antioxidant treatment can help preventing dramatic decline in seminal parameters and sperm function *in vitro*, thus enabling some improvement to selected forms of infertility (Conte et al., 1999). Therefore, the addition of antioxidants into media can be seen as beneficial in preventing motility losses and inhibiting lipid peroxidation. As a consequence, patients treated with antioxidants, in several cases, developed improved fertilizing capacities (Kim and Parthasarathy, 1998).

As mentioned above, the formation of superoxide radicals, is usually the result of electron leakage from mitochondrial electron transport chain due to uncoupled oxidative phosphorylation (Halliwell and Gutteridge, 1999). Therefore there is a relationship between species differences in free radical production in spermatozoa and their rate of oxidative metabolism. The rate of oxidative metabolism in chicken spermatozoa is very similar to that in turkey spermatozoa although turkey spermatozoa are more dependent on oxidative metabolism than fowl spermatozoa to maintain optimal ATP levels (Wishart, 1982). In this respect, the lower unsaturation rate of turkey sperm lipid could be an advantage in terms of prevention of lipid peroxidation. However, the antioxidant enzyme activities in turkey spermatozoa appear to be below that observed in fowl sperm (Surai et al., 1998). Up to date there are no data available on the metabolic comparisons between other avian species.

Whilst the ratio of PUFAs to antioxidants in spermatozoa is a very important determinant of their survival *in vitro*, this could also be a factor in survival of spermatozoa in the oviduct. The unique feature of avian reproduction is spermatozoa storage within oviducal 'sperm storage tubules' (SST) over prolonged period up to several weeks (Bakst et al., 1994). Therefore the maintenance of membrane stability and prevention of lipid peroxidation at body temperature (39-41°C) sperm storage could be a strategy for avian species. In this respect the level of lipid unsaturation and fatty acid profile of avian spermatozoa are different from those of mammals. Thus, turkey spermatozoa characterized by the lowest degree of lipid unsaturation have the longest fertile period in the SST (up to 2 months). Therefore an antioxidant role for the SST has been proposed (Surai et al., 1998).

With regard to species-specific features of fatty acid profiles and antioxidant protection of avian spermatozoa a number of questions remain unanswered. For example, duck spermatozoa containing the highest proportions of PUFA and characterised by the highest peroxidizability index of their lipids had vitamin E concentration much lower compared to chickens (Surai et al., 2000). However the duck spermatozoa susceptibility to peroxidation was similar to that in chicken spermatozoa (Surai et al., 2000) emphasising an important role of increased activities of antioxidant enzymes in the duck spermatozoa (Surai et al., 1998). Furthermore, there are also some anatomical differences in the reproductive system of duck allowing them extra-protection against lipid peroxidation in the oviduct tract. For example, during copulation in fowl and turkey, semen is deposited in the lower portion of the vagina and spermatozoa must migrate through most of luminal portion of the vagina before reaching the storage site. Therefore this migration is associated with exposure to the oxygenated environment of the vaginal mucosa. In contrast, in duck, due to anatomical difference in reproductive system (very developed penis; Sauveur and de Carville, 1990), ejaculated semen is deposited in the upper vagina, which is isolated from direct contact with ambient air. Therefore, the antioxidant defense in duck spermatozoa may not, in the natural situation, be as crucial as in the fowl and turkey (Surai et al., 2000). It is interesting that in terms

of metabolism of spermatozoa, the anaerobically active lactate dehydrogenase (LDH) isoenzymes 4 and 5 were predominant in drake and gander spermatozoa, whilst LDH1 and 2, which are typical of aerobically metabolizing tissues, were predominant in fowl spermatozoa (Surai, 1991a). Furthermore, hexokinase and phosphofructokinase activities were higher in drake and gander spermatozoa than in those found in the fowl and turkey (Surai, 1991; 1991a). These characteristics suggest that waterfowl spermatozoa may be adaptive to storage under anaerobic conditions, provided that a glycolytic substrate is present. Therefore, metabolic differences between duck and fowl spermatozoa may be related to the differences in strategy of their antioxidant defence.

MODULATION OF FATTY ACID COMPOSITION AND ANTIOXIDANT CAPACITY OF SEMEN

PUFA

As reported above, the fatty acid composition of avian semen has species-specific features showing different profiles irrespective of their similarity for the dietary supply of fatty acids (Surai et al., 1998). Meanwhile, at most occasions the chemical composition of the diet remains the major determinant of the fatty acid composition of animal tissues including spermatozoa. Therefore changes in the dietary composition may be effective in adapting the fatty acid profile of avian spermatozoa. For example, diet supplemented with fish oil induced a significant increase in the proportion of 22:6n-3 in the spermatozoan phospholipid in parallel with an equivalent decrease in the proportions of 20:4n-6 and 22:4n-6 (Kelso et al., 1997; Surai et al., 2000a). Similarly in the phospholipid fraction, the DHA proportion increased from 3.8 up to 9.8% at 40 weeks of age and from 5.1 to 9.1% at 58 weeks of age as a result of inclusion of 3% tuna oil into the cockerel diet (Kelso et al., 1997). The major increase in DHA proportion (from 6.1 to 16.1%) was observed in the PE fraction. At the same time in the PC fraction the DHA proportion increased from 1.1 up to 4.1%. There was also increase in the DHA proportion in the cholesterol ester fraction (from 3.7 up to 9.2%) and a tendency ($p>0.05$) of increasing the DHA proportion in the triacylglycerol fraction (from 2.8 up to 5.3%). Nevertheless the proportion of 22:6n-3 that was achieved in the phospholipid fraction of the chicken spermatozoa as a result of fish oil feeding fell far short of that which is routinely observed for mammalian semen (Scott, 1973).

The confirmation of the limited capacity of spermatozoa to include DHA came from another experiment where tuna oil supplementation was increased up to 5% (Surai et al., 2000a). Under these conditions the DHA concentration of the phospholipid fraction of the spermatozoa increased from 4.6% up to 12.2% at 60 weeks of age. Such an increase in DHA content was associated with a compromised antioxidant defence, since levels of vitamin E significantly decreased while the susceptibility to peroxidation increased. A dietary supplementation with DHA was beneficial for males in maintaining testis mass and sperm production at the end of the reproductive period (Surai et al., 2000a). Similar changes in the fatty acid profiles have also been observed in the tissues of fowl males fed a similar diet (Surai and Sparks, 2000). For example, as a result of tuna oil dietary supplementation (3%), the proportions of DHA in the phospholipid fraction increased from 13.0 to 24.9% in the liver and from 2.5 to 6.0% in the testes (Surai and Sparks, 2000). Inclusion in the cockerel's diet of Arasco oil (rich in 20:4n-6) was associated with an increase in proportions of 22:4n-6 in the phospholipid fractions of the testes (from 15.6 to 18.0%) and liver (from 1.0 to 3.9%) (Surai et al., 2000a). Again, increased DHA concentration in the tissues was associated with decreased vitamin E concentration and increased tissue susceptibility to lipid peroxidation (Surai and Sparks, 2000). Furthermore, an enrichment of fowl spermatozoa with n-3 fatty acids was associated with increased fertilizing ability (Blesbois et al., 1997, 1997a). However, semen produced under these conditions became more sensitive to *in vitro* manipulation, including deep freezing, when fertilizing ability decreased from 32.1 to 8.6% (Blesbois et al., 1997a). This might be a result of increased lipid peroxidation in semen. Therefore during lipid manipulation of the spermatozoa an adequate antioxidant protection should be provided, for example, by increased dietary vitamin E supplementation.

Another option for changing the fatty acid profile in fowl spermatozoa was the inclusion of linseed oil (a rich source of 18:3n-3) in the male diet. In this case only a moderate increase in 22:5n-3 was observed at 39 and 54 weeks of age which disappeared at 72 weeks of age (Kelso et al., 1997a). Most notably, no significant changes in the amount of 22:6n-3 were induced by 18:3n-3 dietary supplementation. Therefore a major conclusion from the work was that the fatty acid profile of the spermatozoa of the cockerels exhibited a distinct resistance to dietary manipulation - the sustained feeding of large amounts of 18:3n-3 to the fowl males completely failed to dislodge the ascendancy of n-6 polyunsaturates in the spermatozoa of the birds representing a clear-cut biochemical difference between mammalian and avian species.

Inclusion in the fowl male's diet of high levels of arachidonic acid (28% of total fatty acids) increased the proportion of 22:4n-6 in the phospholipid fraction of spermatozoa only from 22.1 up to 25.2% ($p<0.05$) again showing a marked resistance to dietary manipulation (Surai et al., 2000a). Thus, the fatty acid profiles of spermatozoa result from high degree of selectivity, presumably to maintain the appropriate biophysical properties of the cell

membrane, which in turn, indicates that biochemical changes in this membrane are not just a simple consequence of dietary variation.

Therefore the results of studies of dietary manipulation of lipids (Kelso et al., 1997, 1997a; Blesbois et al., 1997, 1997a; Surai et al., 2000a) clearly showed that the prevalence of n-6 fatty acids in spermatozoa is not a result of dietary n-3 insufficiency but, rather, a reflection of marked differences in the fatty acid metabolism between mammalian and avian species. The functional significance of this n-6/n-3 dichotomy between avian and mammalian spermatozoa may reflect an adaptation to temperature (Kelso et al., 1997). Indeed, the higher body temperature of birds compared to mammals (41°C versus 37°C) (Romijn et al., 1966) requires special mechanisms for spermatozoa to maintain adequate membrane functions including fluidity, flexibility and permeability at this temperature. Therefore the decreased number of double bonds in 22:4n-6 compared to 22:6n-3 could be a way to accommodate such requirements. This suggestion is in agreement with the general view on the effect of PUFA composition on membrane properties (Neuringer et al., 1988). The limited response of spermatozoa to increased dietary supply of arachidonic acid could be a reflection of the requirement in fatty acid composition to maintain physiological functions in spermatozoa. Again, the changes in PUFA profile which took place as a result of tuna oil supplementation were not dramatic and 22:4n-6 and 20:4n-6 remained the major PUFAs of the chicken spermatozoa. It is necessary to underline that 22:4n-6 fatty acid is unique for avian semen and not found in any other tissues in the same proportions. A comparison in fatty acid profiles present in liver and testes strongly suggested that the 22:4n-6 is essential for the maturation of avian spermatozoa and is synthesized within the testis (Cerolini et al., 1997a). Its precursor (20:4n-6) is delivered from the liver. The active synthesis of long-chain PUFAs in the rat testis has also been shown recently (Retterstol et al., 1997).

Selenium

The supplementation of Se in the male diet significantly increased Se-GSH-Px activity in the liver, testes, spermatozoa and seminal plasma (Surai et al., 1998c). This resulted in a significant decrease in the sperm's susceptibility to lipid peroxidation as indicated by data on MDA accumulation as a result Fe-stimulated lipid peroxidation. In this study the increased activity of Se-GSH-Px in sperm obtained from birds fed the Se-supplemented diet was associated with greater protection against lipid peroxidation in stored compared to fresh semen. As GSH-Px in the sperm is considered to be the main enzyme removing peroxides and protecting cells against damage caused by the free radicals and the products of lipid peroxidation *in vivo* (Griveau et al., 1995). The protective effect of Se during sperm storage is also probably associated with this function. The results reveal the importance of GSH-Px in the protection of fowl sperm against lipid peroxidation during storage. Since hydrogen peroxide and lipid peroxides are toxic to spermatozoa (Alvarez et al., 1987; De Lamirande and Gagnon, 1992), GSH-Px plays an important role in protecting cell membrane lipid from peroxidation, thus maintaining the integrity of the cell (Flohe and Zimmermann, 1970). In this respect,it is extremely important that an inducable form of the enzyme (Se-GSH-Px) represents >75% of the total enzymatic activity in spermatozoa and >60% in fowl testes and liver. Also, GSH-Px activity in the testis was less than half that observed in the liver. Similarly in rat testis GSH-Px activity was found to be lower than that observed in liver, heart, kidney or lung (Lei et al., 1995) while phospholipid hydroperoxide glutathione peroxidase appears to play a more important role in rat testis than liver (Cockell et al., 1996). A similar stimulating effect of Se-supplementation on GSH-Px activity in sows has been found at a level of 0.5 mg Se/kg in the diet (Neumann and Bronsch, 1988).

To our knowledge, no specific information exists on the effect of Se supplementation of avian males on functional characteristics of spermatozoa and their fertilizing ability. However, some evidence has been provided from studies on cattle and humans indicating that Se supplementation enhances the *in vitro* motility and oxygen uptake of sperm (Julien and Murray, 1977; Pratt, 1978; Vezina et al., 1996). Furthermore, selenium supplementation has a positive effect on the motility of human sperm in sub-fertile males (MacPherson et al., 1993; Scott et al., 1998). In order to verify the hypothesis that selenium and vitamin E could improve male fertility, nine oligoasthenoteratozoospermic men were supplemented for a period of 6 months with Se and vitamin E. Compared to the baseline period (pre-supplementation) of 4 months, statistically significant increases were observed for Se and vitamin E levels, sperm motility, percent live, and percent normal spermatozoa. These improvements are likely to be "supplementation-dependent," since all of the parameters returned to baseline values during the post-treatment period (Vezina et al., 1996). Se can also effect spermatozoa *in vitro*. For example incubation of ram spermatozoa with selenite, selenocystine or selenomethionine ranging from 10^{-6} to 2.5 X 10^{-5}M significantly improved sperm motility and oxygen consumption (Alabi et al., 1985).

On the other hand, the reproductive system of males is quite sensitive to selenium excess. For example, ingestion of 2 ppm and 4 ppm selenium by the house rat, Rattus rattus, for 5 weeks caused a dose-dependent reduction in its body weight, testicular and cauda epididymidis weights, concentration, motility and percentage of live spermatozoa

with a simultaneous increase in the percentage of their abnormal forms (Kaur and Parshad, 1994).

In this respect a choice of Se sources in the male diet is important. Recently it has been shown that Se is assimilated from organic sources much more efficiently compared to commonly used selenite (Mahan, 1999). This can be translated into higher Se accumulation in the animal tissues and building a selenium reserve, which can be effectively used in stress conditions. It has been suggested (Surai, 2000b) that this difference in Se assimilation has been developed during animal evolution since in nature animals including birds obtain selenium only in organic form and addition of selenium in the diet in the form of selenite is an artificial manipulation even though it helps to solve selenium deficiency problems. Therefore use of organic sources of selenium in the diet is an effective means of enhancement of the efficiency of antioxidant systems of chicken (Surai, 2000a) and probably will be effective in the improvement of semen quality as well. However, the effect of organic selenium on the reproductive performances of avian species needs further investigation.

Vitamin E

Vitamin E concentration in spermatozoa is a reflection of its supplementation into the breeder's diet. For example, inclusion of increased vitamin E doses in the turkey diet was shown to increase α-tocopherol concentration in spermatozoa (Surai, 1983; Surai and Ionov, 1992). To evaluate possible membrane-stabilizing effect of vitamin E on the spermatozoa different approaches were used. First, the release of glutamic-oxalacetic transaminase (GOT) from spermatozoa was considered as a marker of sperm membrane integrity. During sperm storage *in vitro* the GOT activity increased in the medium and decreased in spermatozoa (Surai and Ionov, 1981). Similar changes in GOT activity were later observed in fowl spermatozoa during freeze-thaw procedure (Matsumoto et al., 1985) and a highly significant correlation (r=0.99) was found between GOT activity in seminal plasma and percentage of dead spermatozoa (Bilgili et al., 1985). Another approach was based on use of low concentrations of detergent (Triton X-100) in sperm storage media in order to induce sperm membrane damage. It has been shown that such treatment significantly increased the release of GOT from spermatozoa (Surai, 1989a).

We observed that turkey spermatozoa enriched with vitamin E released less GOT into the medium during sperm storage compared to a control group (Surai, 1982). Even during sperm cryo-preservation a protective effect of increased vitamin E concentration in spermatozoa was observed (Surai, 1988). It was not clear if the stabilizing effect of vitamin E was due to prevention of lipid peroxidation. Therefore, in the next experiment it was shown that an increased level of vitamin E in the turkey spermatozoa was associated with a reduction in susceptibility to Fe^{2+}-induced lipid peroxidation (Surai, 1984). This effect of vitamin E was confirmed with chicken semen (Surai et al., 1997a). Therefore, a prevention of lipid peroxidation could be an important mechanism of stabilizing effect of vitamin E on sperm membranes.

It has been suggested that vitamin E plays a role as a biological stabiliser of sperm plasma membrane (Surai, 1993, 1989, 1999). Increasing the α-tocopherol content of the membrane has been shown to stabilize them, making spermatozoa more resistant to the various 'unnatural' stresses occurring in the course of artificial insemination, short-term storage and cryopreservation (Surai, 1992, Surai and Ionov, 1992). It is notable that in experiments with turkeys, the best results were obtained when vitamin E dietary supplementation was increased from 20 to 80 mg/kg (Surai, 1983, 1991). In this regard it is interesting that recommendations by Cuddy Farms for vitamin E supplementation of breeding toms was 90 mg/kg (Surai, 1999). A positive effect of increased vitamin E supplementation of the gander breeding diet (from 5 to 40 mg/kg diet) associated with increased fertilizing ability of spermatozoa in the course of artificial insemination was also shown (Surai and Ionov, 1992a).

The supplementation of vitamin E in fowl diet at high concentration (200 mg/kg) was associated with an almost two-fold increase in α-tocopherol concentrations in whole fowl semen, spermatozoa and seminal plasma compared with that at 20 mg/kg supplementation (Surai et al., 1997a, 1998c). However, further increase in vitamin E supplementation from 200 up to 1000 mg/kg did not change α-tocopherol concentration in the semen (Surai et al., 1997a), while it increased significantly in the liver and testes. Thus the concentration of α-tocopherol in semen is only partly dependent on its availability in the diet and displays a limited responsiveness to manipulation by dietary means. A possible explanation for such dramatic differences in response between, say, the liver and the spermatozoa may lie in the fact that, whereas the liver is able to accumulate cytoplasmic lipid droplets (Mooney and Lane, 1981) and therefore lipid-soluble vitamins such as α-tocopherol, spermatozoa contain few such inclusions with the consequence that the phospholipid bilayers of the cells' membranes will represent the main site of accumulation of the vitamin. As mentioned above, the maximal concentration of α-tocopherol which can be accumulated in cell membranes is usually very low; less than 1 mol of the vitamin per 1,000 mol of phospholipid (Packer, 1992). Thus there may be a natural biological limitation on the extent to which the α-tocopherol concentration of spermatozoa can be increased.

Meanwhile, the dietary-induced increase of α-

tocopherol content in semen did result in a significant reduction in the susceptibility of semen to lipid peroxidation. In fact, the susceptibility of semen to peroxidation displayed a very high negative correlation (r = -0.998) with the α-tocopherol content of the semen. The susceptibility of testes homogenates to *in vitro* peroxidation was also reduced by the dietary supplementation with α-tocopherol (Surai et al., 1998c). During storage, the susceptibility of spermatozoa to lipid peroxidation significantly increased probably due to the initiation of spontaneous lipid peroxidation (Cecil and Bakst. 1993). In these conditions, the protective effect of vitamin E enrichment of spermatozoa has been clearly demonstrated (Surai et al., 1998c). Thus, even the relatively limited enhancement of semen α-tocopherol content achieved by dietary means was found to produce significant benefits by reducing the susceptibility of the semen to lipid peroxidation. An inverse relationship between susceptibility to peroxidation and α-tocopherol content has previously been demonstrated for microsomal fractions from various mammalian tissues (Kornbrust and Mavis, 1980).

Vitamin E supplementation (200 mg/kg) was shown to be beneficial in preventing lipid peroxidation in sperm as a result of DHA dietary supplementation (Surai et al., 2000a). A similarly protective effect of increased vitamin E supplementation was observed in fowl tissues enriched with DHA (Surai and Sparks, 2000; Surai et al., 2000a). The increase in the proportion of C_{20-22} polyunsaturated fatty acids in the sperm phospholipids resulted from dietary supplementation of the birds with vitamin E (Surai et al., 1997a; 2000a). Hypothetically, this could be an *in vivo* reflection of the reduced peroxidative susceptibility of semen. However, an alternative explanation to this phenomenon could be based on the recent hypothesis of Infante (1999), suggesting that tocopheryl quinone may play an important role as a cofactor in fatty acid elongation. Therefore, increased vitamin E supplementation could alter fatty acid metabolism through this mechanism. Since the antioxidant protection afforded by vitamin E depends on adequate selenium status (Surai, 2000), it would be of considerable interest to evaluate the combined effects of vitamin E + organic selenium supplementation on semen production and quality in avian species.

The positive effects of increased vitamin E supplementation have been also observed in mammalian species. For example, Merino rams given a diet supplemented with 2,500 mg/kg vitamin E exhibited improvements in semen characteristics and acrosomal morphology (Gokcen et al., 1990). Moreover the supplementation of the diet of rams with a much lower level of vitamin E (60 mg/kg) improved the semen characteristics by increasing sperm concentration and motility and decreasing the incidence of sperm abnormalities (Erdinc et al., 1986). Vitamin E supplementation also significantly decreased the level of seminal plasma thiobarbituric acid reacting substances (TBARS) in boars (Brzezinska-Slebodzinska et al., 1995). Supplementation with vitamin E in Pb-exposed rats reduced ROS generation in spermatozoa, prevented loss of sperm motility and capacity of oocyte penetration (Hsu et al., 1998). Furthermore in bulls fed a diet supplemented in vitamin E (0.75 mg/kg body weight), post-thawing motility of spermatozoa was significantly increased (Udala et al., 1995). In rats, dietary vitamin E supplementation did not affect phospholipid hydroperoxide glutathione peroxidase activity in the liver, whereas it raised the enzymatic activity in seminal vesicles by 43% ($p<0.005$) (Lei et al., 1997).

In human, several experiments with infertile patients were carried out to improve their semen quality by means of using high doses of vitamin E. In an experiment carried out in Israel fifteen fertile normospermic male volunteers who had had low fertilizing rates were fed vitamin E (200 mg daily by mouth) for 3 months. After 1 month of experimental treatment the level of peroxidation in the spermatozoa significantly decreased and the fertilization rate per cycle also increased significantly from 19.3 to 29.1. (Geva et al., 1996). In another experiment in the UK to determine the effectiveness of the *in vivo* administration of vitamin E as a treatment for reactive oxygen species-associated male infertility a double-blind randomised placebo cross-over controlled trial was designed (Kessopoulow et al., 1995). Thirty healthy men with high levels of ROS generation in semen and a normal female partner were allocated to two groups according to the blinded randomisation. Each patient received either 600 mg/d of vitamin E (Ephynal, 300 mg tablets; Hoffman-La Roche Ltd., Basele, Switzerland) or identical placebo tablets for 3 months. In this experiment a rise in blood serum vitamin E levels after treatment was accompanied by significant improvement of the *in vitro* function of human spermatozoa as assessed by the zona-binding test.

In Finland, in an open controlled study, the blood and seminal plasma concentrations of α-tocopherol in 15 unselected male volunteers were increased as a result of receiving either 600, 800 or 1200 mg α-tocopherol per day orally for 3 weeks (Moilanen and Hovatta, 1995). Vitamin E levels in seminal plasma of infertile men can be elevated also by oral administration of vitamin E (Moilanen et al., 1993). In Saudi Arabia 110 patients who were classified as asthenospermic received 100 mg vitamin E or placebo three times a day for 6 months (Suleman et al., 1996). Treatment of asthenospermic patients with vitamin E significantly decreased the MDA concentration in spermatozoa and improved sperm motility. Eleven of the 52 treated patients impregnated their spouses; no pregnancies were reported in the spouses of placebo-treated patients.

Ascorbic acid

Fowl males housed under hot and humid tropical conditions showed increased overall quality of ejaculates (semen volume, motile sperm per ejaculate and sperm number per ejaculate) as a result of ascorbic acid supplementation at level of 500 mg/kg (Monsi and Onitchi, 1991). However, spermatozoa motility was not affected. The supplementation of rainbow trout with ascorbyl monophosphate was associated with a prevention of reduction in DHA in their semen and decreased MDA accumulation due to increased ascorbic acid concentration in seminal plasma (Liu et al., 1997). The spermatozoa from supplemented group had the highest motility and lowest decline in fertilizing ability after storage (Ciereszko and Dabrowski, 2000). A protective role of ascorbic acid toward maintaining fish sperm quality was suggested (Ciereszko and Dabrowski, 1996). When the seminal plasma ascorbate concentration in rainbow trout decreased to 7.3 μg/ml a significant decrease of fertilization rate and hatching rate of embryos resulted (Dabrowski and Ciereszko, 1996). Similarly dietary ascorbic acid protects human sperm from endogenous oxidative DNA damage that could affect sperm quality and increase risk of genetic defects (Fraga et al., 1991). In contrast, dietary supplementation with low doses of vitamin C and E did not have any effect on mouse sperm quality, however, high doses of these vitamins decreased number of spermatozoa/mg epididymis and increased the percentage of spermatozoa with misshapen heads (Ten et al., 1997).

CONCLUSIONS

As reported above, a number of experiments indicate that avian spermatozoa contain high concentrations of PUFAs that make them vulnerable to lipid peroxidation, especially during *in vitro* manipulation. In these species, docosatetraenoic (22:4n-6) and arachidonic (20:4n-6) fatty acids are the most vulnerable to lipid peroxidation. In general, lipid peroxidation in semen should be considered as an important cause of decreased sperm quality and reduced fertilizing ability. Excessive free radical generation by spermatozoa could be induced by various factors, including uncoupling oxidation and phosphorylation in mitochondria, the redox cycling of xenobiotics, excessive NADPH oxidase activity, the increased availability of transition metals and, possibly also, a cause of impaired antioxidant protection initiated by dietary deficiencies, age or genetic factors (Aitken, 1999). In this respect the various antioxidant systems of semen play an important role protecting sperm membranes against the damaging effects of free radicals and toxic products of their metabolism. However it should be noted that there are species-specific, age-related differences in the expression of the various antioxidant systems in avian semen. Thus seminal plasma per se is considered to possess reasonable free radical-trapping and antioxidant activities. Furthermore, the inclusion of natural antioxidants into sperm diluents is proven to inhibit lipid peroxidation and improve semen quality during *in vitro* storage. An increase in the DHA concentration in semen is shown to decrease the antioxidant protection of spermatozoa and cause lipid peroxidation. The dietary supplementation of increased vitamin E levels is found to be effective to prevent lipid peroxidation in such cases. In general, vitamin E is considered to be an effective membrane-stabilizing antioxidant of avian semen.

Accordingly in females, the antioxidant function of SST has been suggested and species-specific differences in strategy of antioxidant defence have been described. On the basis of these observations, improving the antioxidant potential of semen could present a major opportunity in order to optimize their fertilizing capacities. Recent observations indicate that the antioxidant/prooxidant balance in avian semen is an important element in purpose of maintaining their membrane integrity and biological functions. The antioxidant system can be suggested to be a crucial element of such a regulation. Meanwhile, it appears that the regulating role of ROS in sperm capacitation and acrosome reaction of several mammalian species has been demonstrated while their role in avian reproduction remains to be elucidated. Clearly there is a need for further research to understand molecular mechanisms of lipid peroxidation and antioxidant protection in avian semen.

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REFERENCES

Aitken, R. J. 1999. The human spermatozoa – a cell in crisis? J. Reprod. Fertil. 115:1-7.

Aitken, J. R. and J. S. Clarkson. 1988. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J. Androl. 9:367-376.

Aitken, J. R., J. S. Clarkson. and S. Fishel. 1989. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol. Reprod. 40:183-197.

Aitken, R. J., D. Harkiss and D. Buckingham. 1993. Relationship between iron-catalysed lipid peroxidation potential and human sperm function. J. Reprod. Fertil. 98:257-265.

Aitken, R. J., D. W. Buckingham and D. Harkiss. 1993a. Use of xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. J. Reprod. Fertil. 97:441-450.

Akesson, B., N. Bellew and R. F. Burk. 1994. Purification of selenoprotein P from human plasma. Biochem. Biophys. Acta. 1204:243-249.

Alabi, N. S., M. A. Beilstein and P. D. Whanger. 2000. Chemical forms of selenium present in rat and ram spermatozoa - In vivo and in vitro studies. Biol. Trace Elem. Res. 76:161–173.

Alabi, N. S., P. D. Whanger and A. S. Wu. 1985. Interactive effects of organic and inorganic selenium with cadmium and mercury on spermatozoal oxygen consumption and motility in vitro. Biol. Reprod. 33:911-919.

Alkan, I., F. Simsek, G. Haklar, E. Kervancioglu, H. Ozveri, S. Yalcin and A. Akdas. 1997. Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: Relationship to seminal plasma antioxidants. J. Urology 157:140-143.

Alvarez, J. G., J. C. Touchstone, L. Blasco and B.T. Storey. 1987. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. J. Androl. 8:338-348.

Alvarez, J. G.and B. T. Storey. 1992. Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethal cryodamage to human sperm during cryopreservation. J. Androl. 13:232-241.

Alvarez, J. G.and B. T. Storey. 1995. Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. Mol. Reprod. Dev. 42:334-346.

Askari, H. A., J. H. Check, N. Peymer and A. Bollendorf. 1994. Effect of natural antioxidants tocopherol and ascorbic acid in maintenance of sperm activity during freeze-thaw process. Arch. Androl. 33:11-15.

Aurich, J. E., U. Schonherr, H. Hoppe and C. Aurich. 1997. Effects of antioxidants on motility and membrane integrity of chilled-stored stallion semen. Theriogenology. 48: 185-192.

Badmaev, V., M. Majeed and R. A. Passwater. 1996. Selenium: a quest for better understanding. Altern. Ther. Health Med. 2:59-62, 65-67.

Beconi, M. T., M. A. Affranchino, L. M. Schang and N. B. Beorlegui. 1991. Influence of antioxidants on SOD activity in bovine sperm. Biochem. Int. 23:545-553.

Bains J. S. and C. A. Shaw. 1997. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res. Rev. 25:335-359.

Bakst M. R., G. J. Wishart and J-P. Brillard. 1994. Oviducal sperm selection, transport and storage in poultry. Poultry Sci. Rev. 5:117-143.

Baumber J., B. A. Ball, C. G. Gravance, V. Medina and M. C. G. Davies-Morel. 2000. The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. J. Androl. 21:895-902.

Bhardwaj A., A. Verma, S. Majumdar and K. L. Khanduja. 2000. Status of vitamin E and reduced glutathione in semen of oligozoospermic and azoospermic patients. Asian J. Androl. 2:225-228.

Behne, D., A. Kyriakopoulos, S. Scheid and H. Gessner. 1991. Effects of chemical form and dosage on the incorporation of selenium into tissue proteins in rats. J. Nutr. 121:806-814.

Behne, D., M. Duk and W. Elger. 1986. Selenium content and glutathione peroxidase activity in the testis of the maturing rat. J. Nutr. 116:1442-1447.

Behne, D., T. Hofer, R. von Berswordt-Wallrabe and W. Elger. 1982. Selenium in the testis of the rat: studies on its regulation and its importance for the organism. J. Nutr. 112:1682-1687.

Behne, D., H. Weiler and A. Kyriakopoulos. 1996. Effects of selenium deficiency on testicular morphology and function in rats. J. Reprod. Fertil. 106: 291-297.

Bell, M., R. Wang, W. J. G. Hellstrom and S. C. Sikka. 1993. Effect of cryoprotective additives and cryopreservation protocol on sperm membrane lipid peroxidation and recovery of motile human sperm. J. Androl. 14: 472-478.

Ben, W. X., M. T. Fu, L. K. Mao, Z. W. Ming and W. W. Xiong. 1997. Effects of various concentrations of native seminal plasma in cryoprotectant on viability of human sperm. Arch. Androl. 39:211-216.

Bilgili, S. F., J. A. Renden and K. J. Sexton. 1985. Fluorometry of poultry semen: Its application in the determination of viability, enzyme leakage and fertility. Poult. Sci. 64:1227-1230.

Bilodeau, J. F., S. Chatterjee, M. A. Sirard and C. Gagnon. 2000. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Molecular Reprod. Dev. 55:282-288.

Bleau, G., J. Lemarbre, G. Faucher, K. D. Roberts and A. Chapdelaine. 1984. Semen selenium and human fertility. Fertil. Steril. 42:890-894.

Blesbois, E., I. Grasseau and J.C Blum. 1993. Effect of vitamin E on fowl semen storage at 4°C. Theriogenology. 39:771-779.

Blesbois, E., I. Grasseau and D. Hermier. 1999. Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5 degrees C. Theriogenology 52:325-334.

Blesbois, E., M. Lessire, I. Grassseau, J. M. Hallouis and D. Hermier. 1997. Effect of dietary fat on fatty acid composition and fertilizing ability of fowl semen. Biol. Reprod. 56:1216-1220.

Blesbois, E., M. Lessire and D. Hermier. 1997a. Effect of cryopreservation and diet on lipids of fowl sperm and fertility. Poult. Avian Biol. Rev. 8:149-154.

Bohm, F., R. Edge, E. J. Land, D. J. McGarvey and T. G. Truscott. 1997. Carotenoids enhance vitamin E antioxidant efficiency. J. Am. Chem. Soc.119:621-622.

Brigelius-Flohe, R. 1999. Tissue-specific functions of individual glutathione peroxidases. Free Rad. Biol. Med. 27:951-965.

Brown, D. V., P. L. Senger, S. L. Stone, J. A. Froseth and W. C. Becker. 1977. Glutathione peroxidase in bovine semen. J. Reprod. Fert. 50: 117-118.

Brzezinska-Slebodzinska E., A.B. Slebodinski, B. Pietras and G. Wieczorek. 1995. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace Element Res. 47: 69-74.

Burk, R. F. and K. E. Hill. 1999. Orphan selenoproteins. Bioessays 21:231-237.

Burk, R. F. and K. E. Hill. 1994. Selenoprotein P. A selenium-rich extracellular glycoprotein. J. Nutr. 124:1891-1897.

Calvin, H. I. 1978. Selective incorporation of selenium-75 into a polypeptide of the rat sperm tail. J. Exp. Zool. 204:445-452.

Calvin, H. I., K. Grosshans, S. R. Musicant-Shikora and S. I. Turner. 1987. A developmental study of rat sperm and testis selenoproteins. J. Reprod. Fertil. 81:1-11.

Castellini, C., P. Lattaioli, M. Bernardini and A. Dal Bosco. 2000. Effect of dietary alpha-tocopheryl acetate and ascorbic acid on

rabbit semen stored at 5 degrees C. Theriogenology. 54:523-533.

Castellini, C., P. Lattaioli, M. Moroni and A. Minelli. 2000a. Effect of seminal plasma on the characteristics and fertility of rabbit spermatozoa. Anim. Reprod. Sci. 63:275-282.

Cerolini, S., A. Maldjian, P. F. Surai and R. C. Noble. 2000. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim. Reprod. Sci. 58:99-111.

Cerolini, S., K. A. Kelso, R. C. Noble, B. K. Speake, F. Pizzi and L. G. Cavalchini. 1997. Relationship between spermatozoan lipid composition and fertility during aging of chickens. Biol. Reprod. 57:976-980.

Cerolini, S., P. Surai, T. Gliozzi, B. Speake, L. Cavalchini and R. Noble. 1998. Antioxidant activities in semen of chicken breeders fed a n-3 and n-6 supplemented diet. 3rd ISSFAL Congress, Abstract Book, Lyon, France , June 1-5, p. 171.

Cerolini, S., P. F. Surai, A. Maldjian, T. Gliozzi and R. Noble. 1997a. Lipid composition of semen in different fowl breeders. Poultry Avian Biol. Rev. 8:141-148.

Cecil, H. C. and M. R. Bakst. 1993. In vitro lipid peroxidation of turkey spermatozoa. Poult. Sci. 72:1370-1378.

Chan, A. C. 1993. Partners in defence, vitamin E and vitamin C. Can. J. Physiol. Pharmacol. 71:725-731.

Chan, A. C., K. Tran, T. Raynor, P. R. Ganz and C. K. Chow. 1991. Regeneration of vitamin E in human platelets. J. Biol. Chem. 266:17290-17295.

Chen, C. S., H. T. Chao, R. L. Pan and Y. H. Wei. 1997. Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm. Biochem. Mol. Biol. Int. 43:291-303.

Chen, J., T. C. Campbell, J. Li and R. Peto. 1990. Diet, life-style and mortality in China. A study of the characteristics of 65 Chinese counties. Oxford: Oxford University Press.

Chu, F. F., J. H. Doroshow and R. S. Esworthy. 1993. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J. Biol. Chem. 268:2571-2576.

Ciereszko A. and K. Dabrowski. 2000. Effect of ascorbic acid supplement in vitro on rainbow trout sperm viability. Aquaculture Int. 8:1-8.

Ciereszko, A., L. Liu and K. Dabrowski. 1996. Effects of season and dietary ascorbic acid on some biochemical characteristics of rainbow trout (Oncorhynchus mykiss) semen. Fish Physiol. Biochem. 15:1-10.

Cockell, K. A., A. R. Brash and R. F. Burk. 1996. Influence of selenium status on activity of phospholipid hydroperoxide glutathione peroxidase in rat liver and testis in comparison with other selenoproteins. J. Nutr. Biochim. 7:333-338.

Comhaire, F. H., A. B. Christophe, A. A. Zalata, W. S. Dhooge, A. M. A. Mahmoud and C. E. Depuydt. 2000. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostagland. Leukotrien. Essential Fatty Acids. 63:159-165.

Comporti, M. 1989. Three models of free radical induced cell injury. Chem. Biol. Interact. 72:1-56.

Connor, W. E., D. S. Lin, D. P. Wolf and M. Alexander. 1998. Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm. J. Lipid Res. 39:1404-1411.

Conte, G., D. Milardi, L. De Marinis and A. Mancini. 1999. Reactive oxygen species in male infertility. Review of literature and personal observations. Panminerva Med 41:45-53.

Conquer, J. A., J. B. Martin, I. Tummon, L. Watson and F. Tekpetey. 1999. Fatty acid analysis of blood serum, seminal plasma and spermatozoa of normozoospermic versus asthenozoospermic males. Lipids. 34:793-799.

Dabrowski, K. and A. Ciereszko.1996. Ascorbic acid protects against male infertility in a teleost fish. Experientia 52:97-100.

Dalton, T. P., H. G. Shertzer and A. Puga. 1999. Regulation of gene expression by reactive oxygen. Annu. Rev. Pharmacol.Toxicol. 39:67-101.

Dalvit, G. C., P. D. Cetica and M. T. Beconi. 1998. Effect of alpha-tocopherol and ascorbic acid on bovine in vitro fertilization. Theriogenology. 49:619-627.

Darin-Bennett, A., A. Poulos and I. G. White. 1974. The phospholipids and phospholipid-bound fatty acids and aldehydes of dog and fowl spermatozoa. J. Reprod. Fertil. 41:471-474.

Dawra, R. K., O. P. Sharma and H. P. Makkar. 1983. Lipid peroxidation in bovine semen. Int. J. Fertil. 28:231-234.

De Lamirande, E. and C. Gagnon. 1992. Reactive oxygen species and human spermatozoa. 1. Effect on the motility of intact spermatozoa and on sperm axonemes. J. Androl. 13:368-378.

Den Boer, P. J., M. Poot, A. Verkerk, R. Jansen, P. Mackenbach and J. A. Grootegoed. 1990. Glutathione-dependent defence mechanisms in isolated round spermatids from the rat. Int. J. Androl. 13:26-38.

Diplock, A. T. 1994. Antioxidants and disease prevention. Mol. Asp. Med. 15:295-376.

Donnelly, E. T., N. McClure and S. E. Lewis. 2000. Glutathione and hypotaurine in vitro: effects on human sperm motility, DNA integrity and production of reactive oxygen species. Mutagenesis. 15:61-68.

Donnelly, E. T., N. McClure and S. E. Lewis. 1999. Antioxidant supplementation in vitro does not improve human sperm motility. Fertil. Steril.72:484-495.

Donnelly, E. T., N. McClure and S. E. Lewis. 1999. The effect of ascorbate and alpha-tocopherol supplementation in vitro on DNA integrity and hydrogen peroxide-induced DNA damage in human spermatozoa. Mutagenesis. 14:505-512.

Donoghue, A. M. and D. J. Donoghue. 1997. Effects of water- and lipid-soluble antioxidants on turkey sperm viability, membrane integrity, and motility during liquid storage. Poult. Sci. 76:1440-1445.

Douard, V., D. Hermier and E. Blesbois. 2000. Changes in turkey semen lipids during liquid in vitro storage. Biol. Reprod. 63:1450-1456.

Elliott, S. J. and S. K. Koliwad. 1997. Redox control of ion channel activity in vascular endothelial cells by glutathione. Microcirculation 4:341-437.

Engel, S., T. Schreiner and R. Petzoldt. 1999. Lipid peroxidation in human spermatozoa and maintenance of progressive sperm motility. Andrologia 31:17-22.

Erdinc, H., H. Gokcen, H. Camas, and E. Cekgul. 1986. Degisik duzeylerde Vit.A ve Vit. E iceren rasyonlarla beslenen koclarin sperma verimi ve ozellikleri uzerinde arastimalar (Sperm

production and traits in rams fed rations with different levels of vitamin A and vitamin E). Veteriner Fakultesi Dergisi, Uludag Universitesi. 5-7:97-101.

Ferrandi, B., F. Cremonesi, A. L. Consiglio, A. Carnevali, and F. Porcelli. 1992. Cytophotometric assay of cytochrome oxidase, lactate dehydrogenase and glucose-6-phosphate-dehydrogenase activities in human peroxidized spermatozoa. Acta Histochemica. 93:363-370.

Ferrandi, B., A. L. Consiglio, A. Carnevali and F. Porcelli. 1992a. Effects of lipid peroxidation on chromatin in rabbit and mouse spermatozoa- a cytochemical approach. Anim. Reprod. Sci. 29, 89-98.

Fietta, P., A. Spisni, L. Lindner and L. Masotti. 1982. Antioxidant activity of bull semen in relation with aging. Bull. Soc. Ital. Biol. Sperm. 58:1079-1085.

Flohe, L., W. A. Gunzler and H. H. Schock. 1973. Glutathione peroxidase: a selenoenzyme. FEBS Lett. 32:132-134.

Flohe, I. and R. Zimmermann. 1970. The role of GSH peroxidase in protecting the membrane of rat liver mitochondria, Biochim. Biophys. Acta. 223:210-213.

Fraga, C. G., P. A. Motchnic, M. K. Shigenaga, H. J. Helbock, R. A. Jacob and B.N. Ames. 1991. Ascorbic acid protects against endogenous oxidative DNA damage un human sperm. Proc. Natl. Acad. Sci. USA. 88:11003-11006.

Freisleben, H. J. and L. Packer. 1993. Free radical scavenging activities, interactions and recycling of antioxidants. Biochem. Soc. Trans. 21:325-330.

Fridovich, I. 1995. Superoxide radical and superoxide dismutases. Ann.Rev. Biochem. 64:97-112.

Froman, D. P. and R. J. Thurston. 1981. Chicken and turkey spermatozoal superoxide dismutase: a comparative study. Biol. Reprod. 24:193-200.

Fujihara, N. and B. Howarth. 1978. Lipid peroxidation in fowl spermatozoa. Poult. Sci., 57:1766-1768.

Fujihara, N. and O. Koga. 1984. Preservation of the production of lipid peroxide in rooster spermatozoa. Anim. Reprod. Sci. 7:385-390.

Fujihara, N. and O. Koga. 1992. Functional differences in sperm membrane among individual male chickens. Proc. 12th Intern. Congr. On Anim. Reprod. Hague. Vol. 1, pp. 452-454.

Gavella, M., V. Lipovac, M. Vucic and B. Rocic. 1996. Superoxide anion scavenging capacity of human seminal plasma. Intern. J. Androl. 19:82-90.

Gavella, M., V. Lipovac, M. Vucic and B. Rocic. 1997. Evaluation of ascorbate and urate antioxidant capacity in human semen. Andrologia. 29:29-35.

Geva, E., B. Bartoov, N. Zabludovsky, J. B. Lessing, L. Lerner-Geva and A. Amit. 1996. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertil. Steril. 66:430-434.

Ghyselinck, N. B., C. Jimenez and J. P. Dufaure. 1991. Sequence homology of androgen- regulated epididymal proteins with glutathione peroxidase in mice. J. Reprod. Fert. 93:461-466.

Gokcen, H., H. Camas, H. Erding, R. Asti, E. Cekgul. and E. Sener. 1990. Studies on the effects of vitamin E and selenium added to the ration on acrosome morphology, enzyme activity and fertility of frozen ram spermatozoa. Doga Turk. Veterinerlik ve Hayvancilik Dergisi. 14:207-218.

Gomez, E., D. S. Irvine and R. J. Aitken. 1998. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. Int. J. Androl. 21:81-94.

Griveau, J. F., E. Dumont, P. Renard, J. P. Callegari and D. LeLannou. 1995. Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. J. Reprod. Fertil. 103:17-26.

Griveau, J. F. and D. Le Lannou. 1994. Effects of antioxidants on human sperm preparation techniques. Int. J. Androl. 17:225-231.

Gutteridge, J. M. C. and B. Halliwell. 1990. The measurement and metabolism of lipid peroxidation in biological systems. Trends Biochem. Sci. 15:129-135.

Halliwell, B. 1994. Free radicals and antioxidants: A personal view. Nutr. Reviews. 52:253-265.

Halliwell, B. and Gutteridge, J. M. C. 1999. Free Radicals in Biology and Medicine. Third Edition. Oxford University Press, Oxford.

Hammerstedt, R. H. 1993. Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: a review of the effect on design of storage preservation systems. Reprod. Fertil. Dev. 5:675-690.

Hansen, J. C. and Y. Deguchi. 1996. Selenium and fertility in animals and man - A review. Acta Vet. Scand. 37:19-30.

Harris, E. D. 1992. Copper as a cofactor and regulator of Copper,Zinc-Superoxide dismutase. J. Nutr. 122:636-640.

Hindshaw, D. B., L. A. Sklar and B. Bohl. 1986. Cytoskeletal and morphologic impact of cellular oxidant injury. Am. J. Pathol. 123:454-464.

Hogg, N. 1998. Free radicals in disease. Semin. Reprod. Endocrin. 16:241-248.

Holben, D. H. and A. M. Smith. 1999. The diverse role of selenium within selenoproteins: A review. J. Am. Diet. Assoc. 99:836-843.

Howarth, B. Jr. 1981. The phospholipid profile of cock spermatozoa before and after in vitro incubation for twenty-four hours at 41°C. Poult Sci. 60:1516-1519.

Howarth, B., D. Torregrossa and W. M. Britton. 1977. The phospholipid content of ejaculated fowl and turkey spermatozoa. Poult Sci. 56:1265-1268.

Hsu, P. C., M. Y. Liu, C. C. Hsu, L. Y. Chen and Y. L. Guo. 1998. Effects of vitamin E and/or C on reactive oxygen species related lead toxicity in the rat sperm. Toxicology. 128:169-179.

Huang,Y. L., W. C. Tseng, S. Y. Cheng and T. H. Lin. 2000. Trace elements and lipid peroxidation in human seminal plasma. Biol. Trace Elem. Res .76:207-215.

Hughes, C. M., S. E. M. Lewis, V. J. McKelvey-Martin and W. Thompson. 1998. The effects of antioxidant supplementation during Percoll preparation on human sperm DNA integrity. Human Reprod. 13:1240- 1247.

Infante, J. P. 1999. A function for the vitamin E metabolite α-tocopherol quinone as an essential enzyme cofactor for the mitochondrial fatty acid desaturases. FEBS Letters 446:1-5.

Irvine, D. S. 1996. Glutathione as a treatment for male infertility. Rev. Reprod. 1:6-12.

Irvine, D. S., J. P. Twigg, E. L. Gordon, N. Fulton, P. A. Milne and R. J. Aitken. 2000. DNA integrity in human spermatozoa: Relationships with semen quality. J. Androl. 21:33-44.

Jackson, M. J., A. McArdle and F. McArdle. 1998. Antioxidant micronutrients and gene expression. Proc. Nutr. Soc. 57:301-305.

Jaeschke, H. 1995. Mechanisms of oxidant stress-induced acute tissue injury. Proc. Soc. Exp. Biol. Med. 209:104-111.

Jones, R. and T. Mann. 1973. Lipid peroxidation in spermatozoa. Proc. Roy. Soc. London. B. 184:103-107.

Jones, R. and T. Mann. 1976. Lipid peroxides in spermatozoa; formation, role of plasmalogen, and physiological significance. Proc. R. Soc. Lond. 193:317-333.

Jones, R. and T. Mann. 1977. Damage to ram spermatozoa by peroxidation of endogenous phospholipids. J. Reprod. Fertil. 50:261-268.

Jones, R. and T. Mann. 1977a. Toxicity of exogenous fatty acid peroxides towards spermatozoa. J. Reprod. Fertil. 50:255-260.

Jones, R., T. Mann, and R. J. Sherins. 1978. Adverse effects of peroxidized lipid on human spermatozoa. Proc. R. Soc. Lond. 201:413-417.

Jones, R., T. Mann and R. Sherins. 1979. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. Fertil. Steril. 31:531-537.

Julien, W. E. and F. A. Murray. 1977. Effect of selenium and selenium with vitamin E on in vitro motility of bovine spermatozoa. In: Proc. Am. Soc. Anim. Sci., 69th Annual Meeting, University of Wisconsin, Madison. P. 174.

Kaludin, I. and I. Dimitrova. 1986. Effect of selenium and DL-methionine on the zinc and tocopherol content of the seminal fluids in rams. Vet. Med. Nauki. 23:41-46.

Kaludin, I. and G. T. Georgiev. 1984. Tocopherol absorption and accumulation in the body of rams. Vet. Med. Nauki. 21:94-97.

Kaludin, I., I. G. Ivanov and I. Dimitrova. 1989. Influence of selenium and methionine on the tocopherol content of ram semen. Veterinarna Sbirka. 87:50-52.

Kantola, M., M. Saaranen and T. Vanha-Perttula. 1988. Selenium and glutathione peroxidase in seminal plasma of men and bulls. J. Reprod. Fert. 83:785-794.

Kaur, R., and V. R. Parshad. 1994. Effects of dietary selenium on differentiation, morphology and functions of spermatozoa of the house rat, Rattus rattus L. Mutat. Res. 309:29-35.

Kelso, K. A., S. Cerolini, R. C. Noble, N. H. C. Sparks and B. K. Speake. 1996. Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. J. Reprod. Fertil. 106:201-206.

Kelso, K. A., S. Cerolini, R. C. Noble, N. H. C. Sparks and B. K. Speake. 1997. The effects of dietary supplementation with docosahexaenoic acid on the phospholipid fatty acid composition of avian spermatozoa. Comp. Biochem. Physiol. 118B:65-69.

Kelso, K. A., S. Cerolini, B. K. Speake L. G. Cavalchini and R. C. Noble. 1997a. Effects of dietary supplementation with α-linolenic acid on the phospholipid fatty acid composition and quality of spermatozoa in cockerel from 24 to 72 weeks of age. J. Reprod. Fertil. 110:53-59.

Kelso, K. A., A. Redpath, R. C. Noble and B. K. Speake. 1997b. Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. J. Reprod. Fertil. 109:1-6.

Kessopoulou, E., H. J. Powers, K. K. Sharma, M. J. Pearson, J. M. Russell, I. D. Cooke and C. L. Barratt. 1995. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil. Steril. 64:825-831.

Kim, J. G. and S. Parthasarathy. 1998. Oxidation and the spermatozoa. Seminars Reprod. Endocrin. 16:235-239.

Kobayashi, T., T. Miyazaki, M. Natori and S. Nozawa. 1991. Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Human Reprod. 6:987-991.

Kohrl, J., R. Brigelius-Flohe, A. Bock, R. Gartner, O. Meyer and L. Flohe. 2000. Selenium in biology: Facts and medical perspectives. Biol. Chem. 381:849-864.

Kornbrust, D. J. and R. D. Mavis. 1980. Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: Correlation with vitamin E. Lipids. 15:315-322.

Kovalski, N. N., E. Delamiranden and C. Gagnon. 1992. Reactive oxygen species generated by human neutrophils inhibit sperm motility – protective effect of seminal plasma and scavengers. Fertil. Steril. 58:809-816.

Ladha, S. 1998. Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. J. Membr. Biol. 165:1-10.

Laudat, A., K. Lecourbe. and A. M. Palluel. 1999. Lipid peroxidation, morphological stress pattern and nuclear maturity of spermatozoon. Ann. Biol. Clin. (Paris) 57:51-56.

Lei, X. G., J. K. Evenson, K. M. Thompson and R. A. Sunde. 1995. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. J. Nutr. 125:1438-1446.

Lei, X. G., D. A. Ross, J. E. Parks and G. F. Jr. Combs. 1997. Effects of dietary selenium and vitamin E concentrations on phospholipid hydroperoxide glutathione peroxidase expression in reproductive tissues of pubertal maturing male rats. Biol. Trace Elem. Res. 59:195-206.

Lenzi, A., L. Gandini, M. Picardo, F. Tramer, G. Sandri and E. Panfili. 2000. Lipoperoxidation damage of spermatozoa polyunsaturated fatty acids (PUFA): Scavenger mechanisms and possible scavenger therapies. Front. Biosci. 5:1-15.

Lenzi, A., L. Gandini, V. Maresca, R. Rago, P. Sgro, F. Dondero and M. Picardo. 2000. Fatty acid composition of spermatozoa and immature germ cells. Molecular Hum. Reprod. 6: 226-231.

Lewis, S. E. M., P. M. Boyle, K. A. Mckinney, I. S. Young and W. Thompson. 1997. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil. Steril. 67:142-147.

Li, K. T. 1975. The glutathione and thiol content of mammalian spermatozoa and seminal plasma Biol. Reprod. 12: 641-646.

Lin, D. S., W.E. Connor, D. P. Wolf, M. Neuringer and D. L. Hachey. 1993. Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid. J. Lipid Res. 34: 491-499.

Liu, L., A. Ciereszko, S. Czesny and K. Dabrowski. 1997. Dietary ascorbyl monophosphate depresses lipid peroxidation in rainbow trout spermatozoa. J. Aquatic Anim. Health. 9:249-257.

Lopes, S., A. Jurisicova, J. G. Sun and R. F. Casper. 1998. Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa. Hum. Reprod. 13:896-900.

MacPherson, A., R. Scott and R. Yates. 1993. The effect of selenium supplementation in subfertile males. In: Proc. 8th Int. Symp. On Trace Elements in Man and Animals. (Ed. M. Anke, D. Meissner and C. F. Mills). Jena, Germany: Verlag Media Touristik. pp. 566-569.

Maddipati, K. R. and L. J. Marnett. 1987. Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. J. Biol. Chem. 262:17398-17403

Maguire, J. J., D. S. Wilson and L. Packer. 1989. Mitochondrial electron-transport tocopheroxyl radical reduction. J. Biol. Chem. 264:21462-21465.

Mahan, D. 1999. Organic selenium: using nature's model to redefine selenium supplementation for animals. In: Biotechnology in the Feed Industry. Proc. of the 15th Annual Symposium. (Ed. T. P.Lyons and K. A. Jacques). Nottingham University Press, Nottingham, UK, pp. 523-535.

Maldjian, A., S. Cerolini, P. F. Surai and B. K. Speake. 1998. The effect of vitamin E, green tea extracts and catechin on the in vitro storage of turkey spermatozoa at room temperature. Poultry Avian Biol. Rev. 9:143-151.

Mann, T. (Ed.) 1981. Male reproduction function and semen. Springer-Verlag Berlin.

Mannella, M. R. T. and R. Jones. 1980. Properties of spermatozoal superoxide dismutase and lack of involvement of superoxides in metal-ion-catalysed lipid peroxidation reactions in semen. Biochem. J. 191:289-297.

Marchaluk, E, M. Persson-Moschos, E. B. Thorling and B. Akesson. 1995. Variation in selenoprotein P concentration in serum from different European regions. Eur. J. Clin. Nutr. 49:42-48.

Marin-Guzman, J., D. C. Mahan, Y. K. Chung, J. L. Pate and W. F. Pope. 1997. Effect of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. J. Anim. Sci. 75:2994-3003.

Mates, J. M. and F. Sanchez-Jimenez. 1999. Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci. 4:D339-D345.

Matsumoto, Y., T. Terada and Y. Tsutsumi. 1985. Glutamic oxalacetic transaminase releases from chicken spermatozoa during freeze-thaw procedure. Poult. Sci. 64:718-722.

McLeod, J. 1943. The role of oxygen in the metabolism and motility of human spermatozoa. Am. J. Physiol 138:512-518.

Meister, A. 1992. On the antioxidant effects of ascorbic acid and glutathione. Biochem. Pharmacol. 44:1905-1915.

Meister, A. and M.E. Anderson. 1983. Glutathione. Ann. Rev. Biochem. 52:711-760.

Michalski, W. 1992. Resolution of three forms of superoxide dismutase by immobilized metal affinity chromatography. J. Chromatogr. Biomed. Appl. 576:340-345.

Monsi, A. and D. O. Onitchi. 1991. Effects of ascorbic acid (vitamin C) supplementation on ejeculated semen characteristics of broiler breeder chickens under hot and humid tropical conditions. Anim. Feed Sci. Technol. 34:141-146.

Mooney, R. A. and M. D. Lane. 1981. Formation and turnover of triglyceride-rich vesicles in the chick liver cell. J. Biol. Chem. 256:11724-11733.

Motoyama, T., M. Miki, M. Mino, M. Takashi and E. Niki, E. 1989. Synergistic inhibition of oxidation in dispersed phosphatidylcholine liposomes by a combination of vitamin E and cysteine. Arch. Biochem. Biophys. 270:655-661.

Moilanen, J., O. Hovatta and L. Lindroth. 1993. Vitamin E levels in seminal plasma can be elevated by oral administration of vitamin E in infertile men. Int. J. Androl.16:165-166.

Moilanen, J. and O. Hovatta. 1995. Excretion of alpha-tocopherol into human seminal plasma after oral administration. Andrologia 27:133-136.

Mukai, K., K. Daifuku, S. Yokoyama and M. Nakano. 1990. Stopped-flow investigation of antioxidant activity of estrogens in solution Biochim. Biophys Acta. 1035:348-352.

Neumann, U. F. and K. Bronsch. 1988. Studies on the optimum supplementation of non gravid and gravid sows. J. Vet. Med. Ser. A. 35:673-682.

Neuringer, M., G. J. Anderson and W. E. Connor. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Ann. Rev. Nutr. 8:517-541.

Niki, E. 1996. α-Tocopherol, In: Cadenas E. and Packer L. eds. Handbook of antioxidants, pp. 3-25. Marcel Dekker.

Niki, E., J. Tsuchya, R. Tanimura and Y. Kamiya. 1982. Regeneration of vitamin E from alpha-chromanoxyl radical by glutathione and vitamin C. Chem. Lett.pp. 789-792.

Nissen, H. P. and H. W. Kreysel. 1983. Polyunsaturated fatty acids in relation to sperm motility. Andrologia. 15:264-269.

Ochsendorf, F. R., R. Buhl, A. Bastlein and H.Beschmann. 1998. Glutathione in spermatozoa and seminal plasma of infertile men. Hum Reprod. 13:353-359.

Oflaherty, C, M. Beconi and N. Beorlegui. 1997. Effect of natural antioxidants, superoxide dismutase and hydrogen peroxide on capacitation of frozen-thawed bull spermatozoa. Andrologia. 29:269-275.

Oldereid, N. B., Y. Thomassen and K. Purvis. 1998. Selenium in human male reproductive organs. Hum. Reprod.13:2172-2176.

Ollero, M., R. Perez-Pe, T. Muino-Blanco and J. A. Cebrian-Perez. 1998. Improvement of ram sperm cryopreservation protocols assessed by sperm quality parameters and heterogeneity analysis. Cryobiology. 37:1-12.

Ollero, A., R. D. Powers and J. G. Alvarez. 2000. Variation of docosahexaenoic acid content in subsets of human spermatozoa at different stages of maturation: Implications for sperm lipoperoxidative damage. Molecular Reprod. Develop. 55:326-334.

Omu, A. E., T. Fatinikun, N. Mannazhath and S. Abraham. 1999. Significance of simultaneous determination of serum and seminal plasma alpha-tocopherol and retinol in infertile men by high-performance liquid chromatography. Andrologia. 31:347-354.

Packer, L. 1992. Interactions among antioxidants in health and disease: Vitamin E and its redox cycle. Proc. Soc. Exp. Biol. Med. 200:271-276.

Packer, L. 1995. Nutrition and biochemistry of the lipophilic antioxidants vitamin E and carotenoids. In: Nutrition, Lipids, Health, and Disease. (Ed. A.S.H. Ong, E. Niki and L. Packer). pp. 8-35. AOCS Press, Illinois.

Packer, L. 1998. alpha-Lipoic acid: a metabolic antioxidant which regulates NF-kappa B signal transduction and protects against oxidative injury. Drug Metab. Rev. 30:245-275.

Palamanda, J. R. and J. P. Kehrer. 1993. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids. 278:427-431.

Palozza, P. and N. I. Krinsky. 1992. β-Carotene and α-tocopherol are synergistic antioxidants. Arch. Biochem Biophys. 297:184-187.

Pasqualotto, F. F., R. K. Sharma, J. M. Potts, D. R. Nelson, A. J. Thomas and A. Agarwal. 2000. Seminal oxidative stress in patients with chronic prostatitis. Urology 55:881-885.

Persson-Moschos, M, G. Alfthan and B. Akesson. 1998. Plasma selenoprotein P levels of healthy males in different selenium status after oral supplementation with different forms of selenium. Eur. J. Clin. Nutr. 52:363-367.

Potts, R. J., L. J. Notarianni and T. M. Jefferies. 2000. Seminal plasma reduces exogenous oxidative damage to human sperm, determined by the measurement of DNA strand breaks and lipid peroxidation. Mutation Res. 447:249-256.

Poulos, A., A. Darin-Bennett and I. G. White. 1973. The phospholipid-bound fatty acids and aldehydes of mammalian spermatozoa. Comp. Biochem. Physiol. 46B:541-549.

Pratt, W. 1978. A study of the effect of in vitro supplementation of sodium selenite on the metabolism of bovine sperm, M.S. Thesis, Ohio State University, Columbus, Ohio.

Ressequire, W. D. and B. L. Hughes. 1984. Phospholipid and cholesterol profiles from chicken seminal components during in vitro storage at 5°C. Poultry Sci. 63:1438-1443.

Retterstol, K., T. B. Haugen and B. Christoferson B. 1997. Metabolism of long chain polyunsaturated fatty acids in rat testis cells. Prostagland. Leukotrien. Essent. Fatty Acids. 57:224.

Rhee, S. G. 1999. Redox signaling: hydrogen peroxide as intracellular messenger. Experim. Mol. Med. 31:53-59.

Rhemrev, J. P. T., F. W. P. C. Van Overveld, G. R. M. M. Haenen, T. Teerlink, A. Bast and J. P .V. Vermeiden. 2000. Quantification of the nonenzymatic past and slow TRAP in a postaddition assay in human seminal plasma and the antioxidant contributions of various seminal compounds. J. Androl. 21:913-920.

Rolf, C., T. G. Cooper, C. H. Yeung and E. Nieschlag. 1999. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum. Reprod. 14:1028-1033.

Romboli, I., M. Marzoni., A. Schiavone and S. Carbas. 2000. Reproductive performance of pheasants fed high vitamin E levels. Proc. XXI World's Poultry Congress, Canada, Montreal, CD-ROM.

Romijn, C., and W. Lokhorst. 1966. Heat regulation and energy metabolism in the domestic fowl. In: Physiology of the Domestic Fowl. (Ed. C. Horton-Smith and E. C. Amoroso) London: Oliver and Boyd; pp. 211-227.

Rotruck, J. T., A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman and W. G. Hoekstra. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-590.

Saaranen, M., U. Suistomaa and T. Vanha-Perttula. 1989. Semen selenium content and sperm mitochondrial volume in human and some animal species. Hum. Reprod. 4:304-308.

Sanchez-Partida, L. G., B. P. Setchell and W. M. C. Maxwell. 1997. Epididymal compounds and antioxidants in diluents for the frozen storage of ram spermatozoa. Reprod. Fertil. Dev. 9:689-696.

Sauveur, B. and H. de Carville. 1990. Le canard de barbarie. Paris: INRA pp.114-115.

Scott, T. M. 1973. Lipid metabolism of spermatozoa. J. Reprod. Fertil. 18:65-76.

Scott, R., A. MacPherson, R. W. S. Yates, B.Hussain and J. Dixon. 1998. The effect of oral selenium supplementation on human sperm motility. Br. J. Urol. 82:76-80.

Sen, C. K. and L. Packer. 2000. Thiol homeostasis and supplements in physical excecise. Am. J. Clin. Nutr. 72(suppl.):553S-669S.

Sevanian, A. and P. Hochstein. 1985. Mechanisms and consequences of lipid peroxidation in biological systems. Ann. Rev. Nutr. 5:365-390.

Sexton, T. J. 1974. Oxidative and glycolitic activity of chicken and turkey spermatozoa. Comp. Biochem. Physiol. 48B:59-65.

Slaweta, R., T. Laskowska and E. Szymanska. 1988. Lipid peroxides, spermatozoa quality and activity of glutathione peroxidase in bull semen. Acta. Physiol. Pol. 39:207-214.

Slaweta, R. and T. Laskowska-Klita. 1985. Glutathione content in the semen of bulls of the lowland black-white breed. Acta Physiol. Pol. 36:107-111.

Smith, D. G., P. L. Senger, J. F. McCutchan and C. A. Landa. 1979. Selenium and glutathione peroxidase distribution in bovine semen and selenium-75 retention by the tissues of the reproductive tract in the bull. Biol. Reprod. 20:377-383.

Smith, R., D. Vantman, J. Ponce, J. Escobar and E. Lissi. 1996. Total antioxidant capacity of human seminal plasma. Human Reprod. 11:1655-1660.

Smutna, M. and O. Synek. 1979. Lipid peroxidation in semen of the boar. Acta Vet. Brno. 48:35-43.

Suleiman, S. A., M. E. Ali, Z. M. Zaki, E. M. el-Malik and M. A. Nasr. 1996. Lipid peroxidation and human sperm motility: protective role of vitamin E. J. Androl. 17:530-537.

Surai, P. F. 1981. Fat-soluble vitamins in turkey sperm. Proc. 2nd Conference of Young Scientists. p. 55-56, Zagorsk, USSR.

Surai, P. F. 1982. The effect of high doses of vitamin E in turkey male diet on the GOT and LDH activity in stored sperm. Nauchno-Tekhnicheskii Byulletin of the Ukrainian Research Poultry Institute. 12:24-29.

Surai, P. F. 1983. Biochemical and functional changes in turkey tissues and sperm as a function of the levels of vitamins E and A in feed. Can. Sci. (PhD) Thesis. Ukrainian Poultry Research Institute, Borky.

Surai, P. F. 1983a. Stabilizing effect of vitamins A and E on turkey sperm membranes. Ptitsevodstvo (Kiev) 36:49-52.

Surai, P. F. 1984. Lipid peroxidation in turkey semen. Ptitsevodstvo (Kiev) 37:58-59.

Surai, P. F. 1988. A protective effect of fat-soluble vitamins during turkey sperm cryopreservation. Abstracts of International Conference on Achievements and Prospects of the Development of Cryobiology and Cryomedicine, Ukraine, Kharkov, pp. 213-214.

Surai, P. F. 1989. Relations between vitamin E concentration in

poultry spermatozoa and some semen biochemical and physiological characteristics. Proc. 8th International Symposium on Current Problems of Avian Genetics, Smolenice, Czechoslovakia, pp. 171-173.

Surai, P. F. 1989a. Detergent treatment of poultry spermatozoa: release of some enzymes. Proc. 8th International Symposium on Current Problems of Avian Genetics, Smolenice, Czechoslovakia, pp. 174-175.

Surai, P. F. 1991. Nutritional and Biochemical Aspects of Vitamins in Poultry. Doctor Sci. (DSc) Thesis. Ukrainian Poultry Research Institute, Borky.

Surai, P. F. 1991a. Comparison of carbohydrate metabolism in semen of cock, turkey, goose and drake. Proc. 9th Internat. Symposium on Current problems of Avian Genetics, Smolenice, Czechoslovakiz, pp. 81-85.

Surai, P. F. 1992. Vitamin E feeding of poultry males. Proc. XIX World's Poultry Congress, Amsterdam The Netherlands, Vol. 1, pp. 575-577.

Surai, P. F. 1999. Vitamin E in avian reproduction. Poult. Avian Biol. Rev. 10:1-60.

Surai, P. F. 2000. Organic selenium: benefits to animals and humans, a biochemist's view. In: Biotechnology in the Feed Industry. Proc. of the 16th Annual Symposium (Ed. T. P. Lyons and K.A. Jacques). Nottingham University Press, Nottingham, UK, pp. 205-260..

Surai, P. F. 2000a. Effect of the selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Brit. Poult Sci. 41:235-243.

Surai, P. F. 2000b. Organic selenium and the egg: lessons from nature. Feed Compounder 20:16-18.

Surai, P. F., E. Blesbois, I.. Grasseau, T. Ghalah, J-P. Brillard, G. Wishart, S. Cerolini and N. H. C. Sparks. 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. 120B:527-533.

Surai, P. F., J-P. Brillard, B. K. Speake, E. Blesbois, F. Seigneurin and N.H.C. Sparks. 2000. Phospholipid fatty acid composition, vitamin E content and susceptibility to lipid peroxidation of duck spermatozoa. Theriogenology 53:1025-1039.

Surai, P. F., S. Cerolini, R.C. Noble, B. K. Speake, N. H. C. Sparks and J. Cloughley. 1997. Spermatozoa Lipids: Protection from peroxidation by α-tocopherol. Prostagland. Leukotrien. Essent. Fatty Acid. 57, 2:263.

Surai, P. F., S. Cerolini, A. Maljian, R. C. Noble and B. K. Speake. 1998a. Effect of lipid peroxidation on the phospholipid and fatty acid composition of turkey spermatozoa: a protective effect of vitamin E. Proc. 50th International Congress on Animal Reproduction, Milano, p. 603.

Surai, P. F., S. Cerolini, G. J. Wishart, B. K. Speake, R. C. Noble and N. H. C. Sparks. 1998b. Lipid and antioxidant composition of chicken semen and its susceptibility to peroxidation. Poultry Avian Biol. Rev. 9:11-23.

Surai, P. F. and I.A. Ionov. 1981. Changes in GOT activity in turkey semen as a result of storage. Nauchno-Tekhnicheskii Byulletin of the Ukrainian Research Poultry Institute. 11:24-30.

Surai, P. and I. Ionov. 1992. Vitamin E in fowl sperm. Proc. 12th Int. Congr. Anim. Reprod., The Huge, The Netherlands, Vol. 1, pp. 535-537.

Surai, P. and I. Ionov. 1992a. Vitamin E in goose reproduction. Proc. 9th Intern. Sympos. Waterfowl, Pisa, Italy, pp. 83-85.

Surai, P. F., I. A. Kostjuk, G. Wishart, A. MacPherson, B. Speake, R.C. Noble, I.A. Ionov and E. Kutz. 1998c. Effect of vitamin E and selenium of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes and liver. Biol. Trace Elem. Res. 64:119-132.

Surai, P. F., E. Kutz, G. J. Wishart, R. C. Noble and B. K. Speake. 1997a. 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. Reprod. Fert. 110:47-51.

Surai, P. F., A. Maldjian, S. Cerolini and N. Sparks. 1998d. Species-specific protective effect of seminal plasma against lipid peroxidation in an in vitro system. Final Programme and Book of Abstracts 8th International Symposium on Spermatology, Montreal, Canada, p. 69.

Surai, P. F., R. C. Noble, N. H. C. Sparks and B. K. Speake. 2000a. Effect of long-term supplementation with arachidonic and docosahexaenoic acids on sperm production in the broiler chicken. J. Reprod. Fert. 120:257-264.

Surai, P. F., R. C. Noble and B. K. Speake. 1996. Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. Biochim. Biochys. Acta. 1304:1-10.

Surai, P. F. and N. H. C. Sparks. 2000. Tissue-specific fatty acid and a-tocopherol profiles in the male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci. 79:1132-1142.

Surai, P. F., G. J. Wishart, A. Maldjian, R. C. Noble and N. H. C. Sparks. 1998e. Lipid peroxidation in avian semen: protective effect of seminal plasma. spermatozoa. Brit. Poultry Sci. 39:S57-S58.

Surai, P. F. and M. S. Zhedek. 1985. Effect of vitamins A and E on turkey sperm quality. Ptitsevodstvo (Kiev) 37:27-34.

Takahashi, K., N. Avissar, J. Whitin and H. Cohen. 1987. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch. Biochem. Biophys. 256:677-686.

Ten, J., F. J. Vendrell, A. Cano and J. J. Tarin. 1997. Dietary antioxidant supplementation did not affect declining sperm function with age in the mouse but did increase head abnormalities and reduced sperm production. Reprod. Nutr. Dev. 37:481-492.

Therond, P., J. Auger, A. Legrand and P. Jouannet. 1996. Alpha-tocopherol in human spermatozoa and seminal plasma: relationship with motility, antioxidant enzymes and leukocytes. Mol. Hum. Reprod. 10:739-744.

Thiele,J. J., H. J. Friesleben, J. Fuchs and F. R. Ochsendorf. 1995. Ascorbic acid and urate in human seminal plasma – determination and interrilationships with chemiluminiscence in washed semen. Human Reprod. 10:110-115.

Thompson, K. H., D. V. Godin D. V. and M. Lee. 1992. Tissue antioxidant status in streptozocin-induced diabetes in rats. Effects of dietary manganese deficiency. Biol. Trace Elem. Res. 35:213-224.

Tramer, F., F. Rocco, F. Micali, G. Sandri and E. Panfili. 1998. Antioxidant systems in rat epididymal spermatozoa. Biol. Reprod. 59:753-758.

Twigg, J., N. Fulton, E. Gomez, D.S. Irvine and R.J. Aitken. 1998. Analysis of the impact of intracellular reactive oxygen species

generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Hum. Reprod. 13:1429-1436.

Udala, J., A. Ramisz, W. Drewnowski, B. Lasota and W. Radoch. 1995. Semen quality of bulls treated with selenium and vitamin E. Jakosc nasienia po podaniu buhajom selenu i witaminy E. Zeszyty Naukowe Akademii Rolniczej w Szczecinie, Zootechnika. 32:57-63.

Upreti, G. C., K. Jensen, J. E. Oliver, D. M. Duganzich, R. Munday and J.F. Smith. 1997. Motility of ram spermatozoa during storage in a chemically-defined diluent containing antioxidants. Anim. Reprod. Sci. 48:269-278.

Ursini, F., M. Maiorino, M. Valente, L. Ferri and C. Gregolin. 1982. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim. Biophys. Acta 710:197-211.

Ursini, F., M. Maiorino and A. Roveri. 1997. Phospholipid hydroperoxide glutathione peroxidase (PHGPx): more than an antioxidant enzyme? Biomed. Environ. Sci. 10:327-332.

Ursini, F, S. Heim, M Kiess, M. Maiorino, A. Roveri, J. Wissing and L. Flohe. 1999. Dual function of the selenoprotein PHGPx during sperm maturation. Science. 285:1393-1396.

van Overveld, F. W. P. C., G. R. M. M. Haenen, J. Rhemrev, J.P.W. Vermeiden and A. Bast. 2000. Tyrosine as important contributor to the antioxidant capacity of seminal plasma. Chem-Biol. Interact. 127:151-161.

Verma, A. and K. C. Kanwar. 1998. Human sperm motility and lipid peroxidation in different ascorbic acid concentrations: an in vitro analysis. Andrologia 30:325-329.

Vezina, D., F. Mauffette, K. D. Roberts and G. Bleau. 1996. Selenium-Vitamin E supplementation in infertile men - effects on semen parameters and micronutrient levels and distribution. Biol. Trace Element Res. 53:65-83.

Wales, R. G., I. G. White and D. R. Lammond. 1959. The spermicidal activity of hydrogen peroxide in vitro and in vivo. J. Endocrinol. 18:236-244.

Watanabe, T. and A. Endo. 1991. Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. Mutat. Res. 262:93-99.

Wingler, K. and R. Brigelius-Flohe. 1999. Gastrointestinal glutathione peroxidase. BioFactors. 10:245-249.

Wishart, G. J. 1982. Maintenance of ATP concentration in and of fertilizing ability of fowl and turkey spermatozoa in vitro. J. Reprod. Fert. 66:457-462.

Wishart, G. J. 1984. Effects of lipid peroxide formation in fowl semen on sperm motility, ATP content and fertilising ability. J. Reprod. Fertil. 71, 113-118.

Wu, A. S., J. E. Oldfield, L. R. Shull and P. Cheeke. 1979. Specific effect of selenium deficiency on rat sperm. Biol. Reprod. 20:793-798.

Zabludovsky, N., F. Eltes, E. Geva, E. Berkovitz, A. Amit, Y. Barak, D. Har-Even and B. Bartoov. 1999. Relationship between human sperm lipid peroxidation, comprehensive quality parameters and IVF outcome. Andrologia 31:91-98.

Zalata, A. A., A. B. Cristophe, C. E. Depuydt, F. Schoonjans and F. H. Comhaire. 1998. The fatty acid composition of phospholipids of spermatozoa from infertile patients. Mol. Human. Reprod. 4:111-118.