Male Fertility and Lipid Metabolism

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Chapter 15

Comparative Aspects of Lipid Peroxidation and Antioxidant Protection in Avian Semen

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Abstract

Avian spermatozoa are characterized by high proportions of polyunsaturated fatty acids (PUFA) in the phospholipid fraction of their membranes. This feature is associated with increased susceptibility of spermatozoa to free radical attack and lipid peroxidation. Therefore antioxidant protection is a vital element in maintaining sperm membrane integrity, motility, and fertilizing ability. It has been suggested that natural antioxidants (vitamin E, ascorbic acid, and glutathione) together with antioxidant enzymes (superoxide dismutase and glutathione peroxidase) build an integrated antioxidant system in avian semen capable of protecting it against free radicals and toxic products of their metabolism. There are species-specific, age-related differences in the expression of antioxidant systems in avian semen. The antioxidant/prooxidant balance in avian semen is an important determinant of membrane integrity and functions, including sperm viability and fertilizing capacity. Vitamin E is considered to be an effective membrane-stabilizing antioxidant of avian semen and dietary supplementation of this vitamin is found to be effective in preventing lipid peroxidation in the spermatozoa. Enhancement of the antioxidant capacity of semen could present a major opportunity for improving male fertility. A regulating role of reactive oxygen species (ROS) in sperm capacitation and the acrosome reaction in mammalian species has been demonstrated; but their role for avian species reproduction remains to be elucidated.

Introduction

Avian spermatozoa are unique in structure and chemical composition and are characterized by high proportions of polyunsaturated fatty acids (PUFA) in the phospholipid fraction of their membranes (1). This feature of these highly specialized cells is a reflection of the specific needs of their membranes for high levels of fluidity and flexibility, which are necessary for sperm motility and fusion with the egg. This functional advantage conferred by PUFA is, however, associated with disadvantages in terms of the susceptibility of sperm to free radical attack and lipid peroxidation. Therefore,

antioxidant protection is a vital element in maintaining sperm membrane integrity, motility, and fertilizing ability. It has been suggested (1) that natural antioxidants (vitamin E, ascorbic acid, and glutathione) together with antioxidant enzymes (superoxide dismutase and glutathione peroxidase) build an integrated antioxidant system in avian semen capable of protecting it 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 are important points for consideration (2).

Mechanisms and Consequences of Lipid Peroxidation in Avian Semen

The most important effect of free radicals on cellular metabolism is due to their participation in lipid peroxidation reactions. The first step of this process is called the initiation phase, during which carbon-centered free radicals are produced from a precursor molecule. For example, a polyunsaturated fatty acid (LH) is converted to a radical by abstraction of a hydrogen atom:

$$LH \xrightarrow{\text{Initiator}} L^* \qquad [1]$$

In the presence of oxygen these radicals (L*) react quickly with the oxygen to produce peroxyl radicals, thereby starting the next stage of lipid peroxidation, the propagation phase:

$$L^* + O_2 \longrightarrow LOO^* \qquad [2]$$

At this stage a relatively unreactive carbon-centered radical (L*) is converted to a highly reactive peroxyl radical. A resultant peroxyl radical can attack any available peroxidazable material producing hydroperoxide (LOOH) and a new carbon-centered radical (L*):

$$LOO^* + LH \longrightarrow LOOH + L^* \qquad [3]$$

Therefore lipid peroxidation is a chain reaction and a potentially large number of cycles of peroxidation could cause substantial damage to cells. In membranes, the peroxidazable material is represented by PUFA. It is generaliy accepted that the susceptibility of PUFA to peroxidation is proportional to the number of double bounds in the molecule. Therefore docosahexaenoic fatty acid (DHA, 22:6n-3), docosatetraenoic fatty acid (DTA, 22:4n-6), and arachidonic acid (AA, 20:4n-6) are among major substrates for peroxidation in the sperm membrane. It is necessary to underline that the same PUFA are responsible for maintenance of physiologically important membrane

properties, including fluidity and permeability. Therefore, as a result of lipid peroxidation within the biological membranes, their structure and functions are compromised. Since reaction 3 is the rate-limiting step of this chain reaction, any substance that can reduce the concentration of peroxyl radicals will limit lipid peroxidation (3). The main biological chain-breaking antioxidants, vitamin E, vitamin C, and glutathione, act at this step.

It is interesting that toxicity of oxygen free radicals to human spermatozoa was reported more than 55 years ago (4), and the toxic effect of H_2O_2 on chicken semen was shown by Wales *et al.* (5). However, major attention to this subject came in 1970 after publication of several milestone papers by Jones and Mann based on the results of experiments conducted in the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry, University of Cambridge (6–11). These publications clearly showed that lipid peroxidation

- Takes place in mammalian spermatozoa
- Causes decline in motility of spermatozoa
- Irreversibly abolishes the fructolytic and respiratory activity of spermatozoa
- Increases release of intracellular enzymes from spermatozoa into medium
- Is the major biochemical cause of sperm senescence under storage conditions *in vitro*
- Causes predominant oxidation of 22:6n-3 and 20:4n-6 fatty acids

Furthermore, those authors also showed that the susceptibility of spermatozoa to peroxidation was increased in cells damaged prior to incubation and that peroxidized PUFA added to a washed sperm suspension immobilized the spermatozoa rapidly and permanently. Those publications presented results obtained with ram and human semen. However, results on lipid peroxidation in other mammalian species have also been published, including boar (12–15), bull (16–20), buffalo (21), rabbit (22), and horse (23,24). Furthermore, lipid peroxidation in human semen has been studied further in detail and several comprehensive reviews have discussed their findings (25–29). The conclusion is that lipid peroxidation in mammalian semen is considered to be one of the most important factors causing infertility in man as well as causing decreased sperm quality during the storage of semen from farm animals.

Research on lipid peroxidation in avian semen started much later in comparison to mammalian species. This is probably because such research has largely been driven by a practical need to understand molecular mechanisms of semen deterioration during storage for further artificial insemination. In poultry production, however, artificial insemination was introduced several decades later than for mammalian species, and its practical usage is related mainly to turkey production. Therefore, a publication by Fujihara and Howarth showing that, during incubation at 41°C, chicken spermatozoa produced a thiobarbituric acid-reactive product and that susceptibility to peroxidation was enhanced by the addition of ascorbate was a starting point in research related to lipid peroxidation in avian semen (30). The authors concluded that chicken spermato-

zoa, following ejaculation and exposure to air, could undergo peroxidation with a consecutive decrease in their viability. The conclusion was very important for future improvement of techniques for avian semen storage *in vitro*. Further evidence for lipid peroxidation in avian semen was presented by Wishart (31), who found that the formation of high concentrations of malondialdehyde (MDA) during a 5-hour aerobic incubation of chicken semen was associated with a partial or complete loss of fertilizing ability. Most importantly, the fertilizing ability of samples that produced low or negligible concentrations of MDA remained unimpaired (31). Semen samples incubated under anaerobic conditions produced only a negligible amount of MDA. There was a considerable (70-fold) variability between individual males in relation to MDA production (31). This could be explained as a result of compositional and functional differences in sperm membranes among individual male chickens (32).

Independently of the work conducted in the United Kingdom by Wishart (31), investigation of lipid peroxidation in turkey semen was started approximately at the same time in Ukraine (33,34). In particular, an induced (by Fe^{2+}) lipid peroxidation in turkey semen was used to assess semen susceptibility to lipid peroxidation since the initial level of peroxides in fresh or even stored semen was shown to be comparatively low. At that time an idea of the possible protective effects of natural antioxidants in the male diet on the lipid peroxidation in the semen was developed (34). In particular, it was shown that dietary supplementation of male turkeys with vitamin E was associated with a significant decrease in semen susceptibility to lipid peroxidation. This work was further developed by Cecil and Bakst (35), who showed that during aerobic storage of turkey spermatozoa, lipid peroxidation was time- and temperature-dependent. The authors also suggested that turkey spermatozoa are more sensitive to lipid peroxidation than semen from other species. It seems likely that lipid peroxidation in semen is also age dependent. For example, Donoghue and Donoghue (36) reported that MDA concentrations were 10-fold higher in semen from older turkey males (56 wk of age) than from younger ones (30 wk of age). This is in agreement with the data of Kelso *et al.* (37), which indicates a fall in the antioxidant defense (activity of glutathione peroxidase, GSH-Px) of chicken spermatozoa between 25 and 60 weeks of age. Accumulation of thiobarbituric acid reactive substances (TBARS) in duck semen as a result of lipid peroxidation has also recently been described (38). It seems likely that MDA accumulation is associated with mid-piece abnormality in human spermatozoa (39) and with a decrease in the fertilizing capacity of chicken (31) and turkey (35) spermatozoa.

The molecular mechanisms of lipid peroxidation in avian semen have received little attention. Recently, it has been shown that during sperm storage, lipid peroxidation is associated with a significant decrease in PUFA concentration in spermatozoa. In particular, the main PUFA in chicken semen (22:4n-6) was most susceptible to peroxidation. Its proportion in the phospholipid fraction was significantly decreased as a result of incubation of chicken sperm for 12 h at 20°C (Fig. 15.1; 40). The inclusion of a promotor of lipid peroxidation (Fe^{2+}) in the incubation medium further increased the rate of lipid peroxidation, significantly decreasing the proportions of not only 22:4n-6

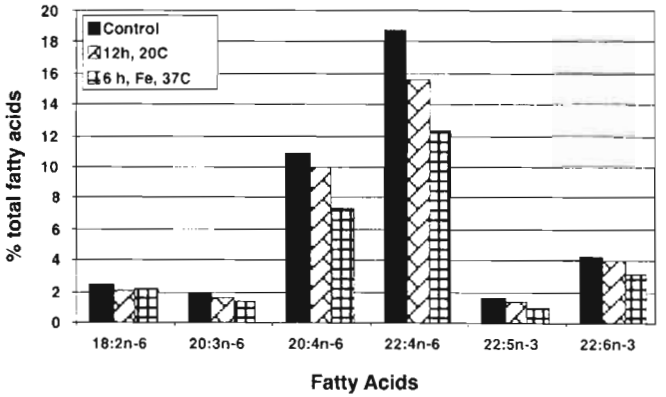

Fig. 15.1. Effect of lipid peroxidation on main PUFA in chicken spermatozoa. Chicken semen was incubated 12 h at 20°C or 6 h at 37°C in the presence of iron. Adapted from Surai *et al.,* 1998.

but also 20:4n-6, 22:5n-3, and 22:6n-3 in the phospholipid fraction of the spermatozoa. The confirmation of the suggestion that the loss of PUFA was due to peroxidation came from the data showing simultaneous accumulation of TBARS in the semen (40). Recently, it has been shown that the total lipid content, the proportion of total phospholipids, and the levels of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (Sph) were significantly decreased in chicken semen during *in vitro* storage, and this was associated with a reduction in the proportion of motile, viable, and morphologically normal cells (41). Similarly, in turkey spermatozoa incubated at 37°C in the 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 (42,43). Storage of diluted turkey semen for 48 h at 4°C was also associated with a decrease in total phospholipid content, PC, and, to a lesser extent, Sph, phosphatidylserine (PS), and phosphatidylinositol (PI) (44). The significance of the decreased concentration of these phospholipids needs further investigation. However, PS appears to be an important phospholipid fraction in avian spermatozoa, having the highest degree of unsaturation (38,45), decreasing during aging (46), and showing a significant positive correlation with the fertilizing ability of chicken semen during the reproductive cycle (47).

As a result of lipid peroxidation in turkey spermatozoa due to a 1-h incubation at 37°C in the presence of Fe^{2+}, there was a significant decrease in the levels of the main PUFA, 22:4n-6, 20:4n-6, 22:6n-3, and 22:3n-9 (Fig. 15.2), and in proportions of PE and PS fractions (Fig. 15.3). Inclusion of vitamin E into the incubation medium decreased PUFA peroxidation and prevented decline in PE and PS fractions in turkey spermatozoa (Fig. 15.2 and 15.3). 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 (48). Therefore, the mechanisms by which reactive oxygen species

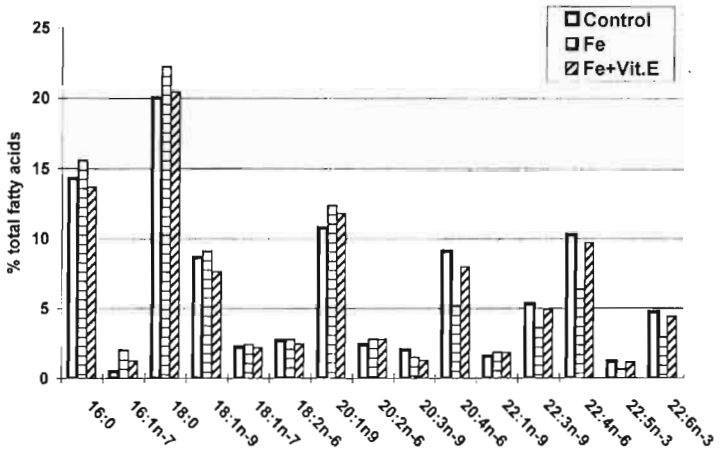

Fig. 15.2. Effect of lipid peroxidation and vitamin E on fatty acid profile of turkey spermatozoa. Turkey semen was incubated in presence of Fe^{2+} or Fe^{2+} + vitamin E at 37°C. Adapted from Maldjian *et al.*, 1998.

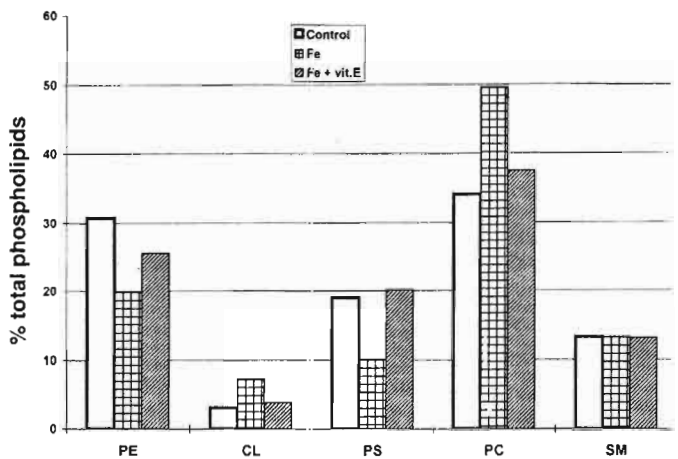

Fig. 15.3. Effect of lipid peroxidation and vitamin E on phospholipid composition of turkey spermatozoa. Turkey semen was incubated in presence of Fe^{2+} or Fe^{2+} + vitamin E at 37°C. Adapted from Maldjian *et al.*, 1998.

(ROS) disrupt sperm function probably involve the peroxidation of PUFA in the sperm plasma membrane. For example, it has been shown that in human spermatozoa, lipid peroxidation damages the cell plasma membrane, leading to loss of cytoplasmic components and hence to cell death—a process that is considered to play an important

role in the pathophysiology of male infertility (39). A negative correlation between MDA production and sperm motility was observed in human semen (49). However, sperm storage at refrigeration temperature is not always associated with lipid peroxidation. For example, in contrast to the above-mentioned observations, storing turkey semen for 48 h at 4°C did not significantly affect the fatty acid profile nor the level of free cholesterol, but the motility, viability, and morphological integrity of spermatozoa significantly decreased (44).

However, lipid peroxidation is not restricted to damage to membranes, and other detrimental consequences for cell metabolism have been described as well (1):

- chromatin destabilization
- marked alterations in the DNA-protein complex
- changes in the activities of various enzymes, including cytochrome oxidase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase
- disruption of mitochondria functions
- inhibition of the synthesis of DNA, RNA, and proteins
- increase of DNA fragmentation
- modification of the cytoskeleton
- alteration in the sperm axoneme
- inhibition of sperm-oocyte fusion

It is necessary to stress that most of the studies on mechanisms and consequences of lipid peroxidation have been associated with mammalian (mainly human) spermatozoa with much less emphasis on avian semen. Nevertheless, lipid peroxidation in avian semen without doubt would have similar consequences. For example, H_2O_2 and organic hydroperoxides had toxic effects on avian sperm motility (Fig. 15.4). On a molar basis, H_2O_2 was about 3 times more powerful in terms of decreasing spermatozoa motility than cumene hydroperoxide. The difference in the toxicity is probably due to higher permeability of plasma membranes for H_2O_2 than for organic hydroperoxides. At the same time, the susceptibility of chicken spermatozoa to H_2O_2 toxicity was much higher than that in mammalian spermatozoa (5).

To understand species-specific differences in lipid peroxidation in spermatozoa, it is necessary to consider an antioxidant/pro-oxidant balance in semen. For example, duck spermatozoa are characterized by increased unsaturation of lipids and decreased vitamin E concentration (Fig. 15.5) in comparison to chicken spermatozoa (38). However, the susceptibility of duck spermatozoa to peroxidation was not different from that of chicken spermatozoa (Fig. 15.6).

Antioxidant Protection in Avian Semen

The antioxidant protection in avian semen is poorly characterized. Recently, it has been suggested that the antioxidant system of the spermatozoa includes three major levels of antioxidant defense (1,50) responsible for maintenance of spermatozoan functions in various stress conditions, including sperm dilution, storage, and deep freezing. Superoxide dismutase (SOD) together with GSH-Px and metal-binding pro-

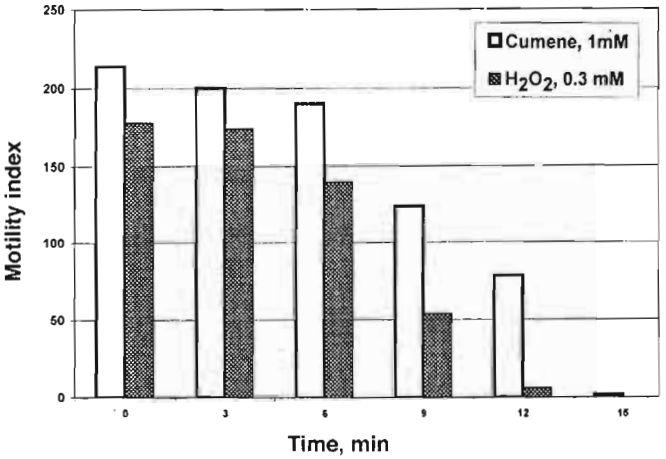

Fig. 15.4. Effect of cumene hydroperoxide or hydrogen peroxide on chicken semen motility.

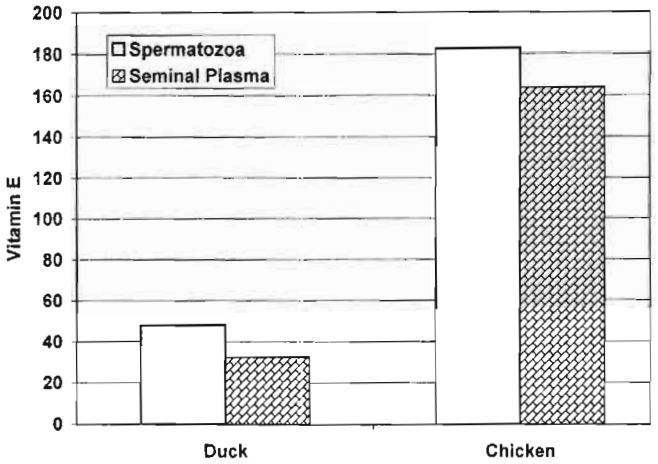

Fig. 15.5. Vitamin E concentration in chicken and duck spermatozoa (ng/10^9) and seminal plasma (ng/ml). Adapted from Surai *et al.*, 2000.

teins comprise the first level of antioxidant defense responsible for prevention and restriction of free radical formation (1). However, the first level of antioxidant defense is not sufficient to prevent the initiation of lipid peroxidation.

Natural antioxidants (vitamin E, ascorbic acid, glutathione), together with additional actions of GSH-Px, build the second level of antioxidant defense dealing with prevention and restriction of chain formation and propagation. Lipid peroxidation can

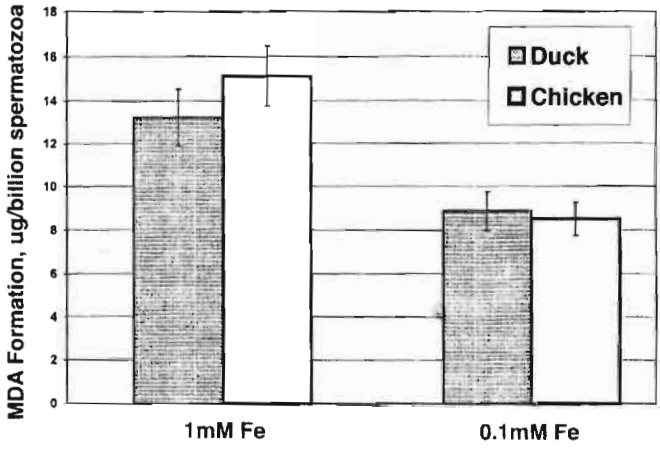

Fig. 15.6. Malondialdehyde formation in duck and chicken semen as a result of Fe-stimulated lipid peroxidation. Adapted from Surai *et al.*, 2000.

be kept under control until these antioxidants are used up and the chain reaction becomes uncontrolled, resulting in damage to cellular constituents and structures.

The third level of defense is based on the enzymatic system responsible for repair or/and removal of damaged molecules from the cell. It seems likely (51) that this level of antioxidant defense in the spermatozoa is either not present or is very inefficient.

First Level of Antioxidant Defense in Avian Semen: Superoxide Dismutase (SOD)

Since the superoxide radical is the main free radical produced under physiological conditions in the cell (52), superoxide dismutase (SOD; EC 1.15.1.1) is considered to be the main element of the first level of intracellular antioxidant defense (50). This enzyme dismutates the superoxide radical in the following reaction:

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

There are three different forms of this enzyme in mammalian and avian species (53). The main form is Mn-SOD, which is located in mitochondria (54), a prime site of superoxide radical production (55). 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 (56).

The second form of the enzyme, Cu,Zn-SOD, is located in the cytosol. The third SOD, so-called extra-cellular SOD is a secretory, Cu,Zn-enzyme found in the interstitial spaces of tissues and in extra-cellular fluids (54). A fourth form of the enzyme, Fe-SOD, is present in various bacteria, blue-green algae, and protozoa but is not found in

animal tissues (53). Cu,Zn-SOD was purified from chicken liver and shown to have two subunits with a molecular weight of 16,000 for each (57).

In spite of the importance of SOD in protecting cells against lipid peroxidation, its activity in avian semen has received only limited attention. For example, when a comparative study of SOD activity was conducted in spermatozoa from boar, rabbit, stallion, donkey, ram, bull, man, and chicken, it was shown that donkey sperm had the highest and chicken spermatozoa the lowest activity (58). Subsequently, turkey spermatozoa were shown to contain even lower SOD activity than chicken spermatozoa and it was suggested that, due to inadequate SOD activity, lipid peroxidation may be a significant factor in poor semen quality and lowered fertility in the turkey (59). The total SOD activity of human seminal plasma was 20 times higher than in blood plasma; human spermatozoa also contained exceptionally large amounts of Cu,Zn-SOD but little Mn-SOD (60).

Our recent data indicate that in seminal plasma of five avian species, only Cu,Zn-SOD was detected (Fig. 15.7 and 15.8; 40,61). There are species-specific differences in SOD activity in the seminal plasma, with the highest SOD activity recorded in turkey and guinea fowl and the lowest activity in duck (Fig. 15.8). Total antioxidant activity of the seminal plasma was also the highest in turkey (Fig. 15.8). In the spermatozoa both forms of SOD are expressed with significant species-specific differences (Fig. 15.7). For example, in goose spermatozoa, the activity of Cu,Zn-SOD was more than twice that of Mn-SOD. An opposite distribution between different forms of SOD was found in guinea fowl, where Mn-SOD was more than twofold higher compared to Cu,Zn-SOD (Fig. 15.9). In the chicken, about 67% of total semen SOD activity was detected in the spermatozoa and only 33% in the seminal plasma (40). The biological meaning and physiological consequences of such species-specific differences in SOD activity and distribution need further clarification.

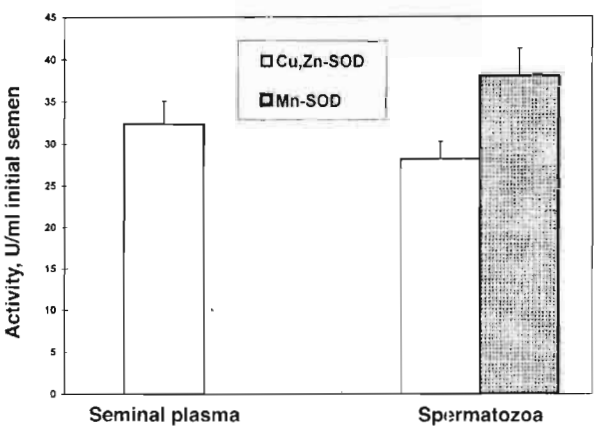

Fig. 15.7. Distribution of SOD in chicken spermatozoa and seminal plasma. Adapted from Surai *et al.*, 1998a.

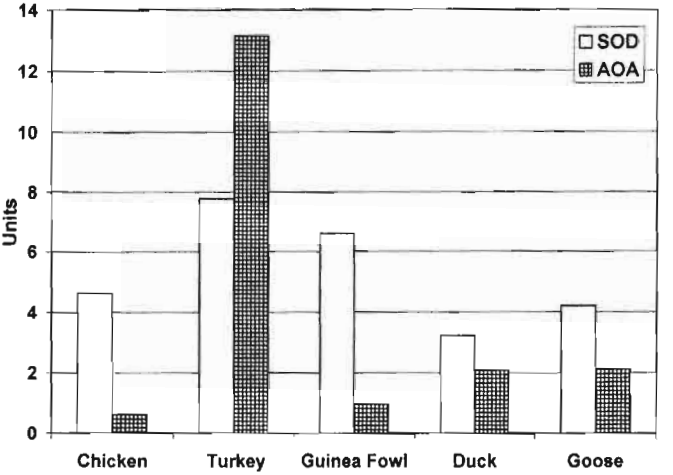

Fig. 15.8. Superoxide dismutase and total antioxidant activity in avian seminal plasma. Adapted from Surai *et al.*, 1998a.

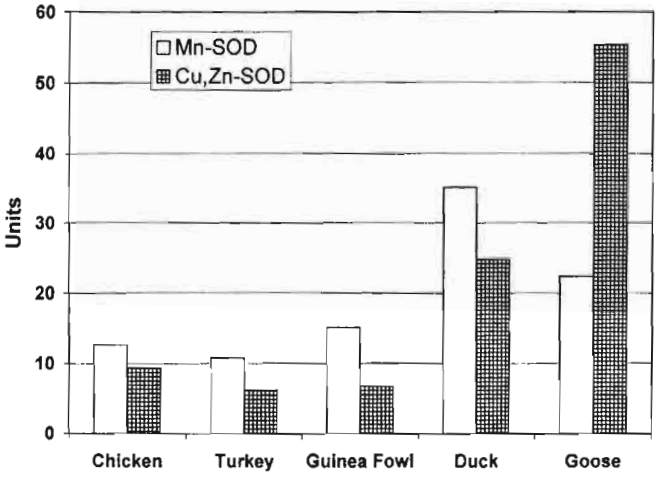

Fig. 15.9. Superoxide dismutase activity in avian spermatozoa. Adapted from Surai *et al.*, 1998a.

Se and GSH-Px

The essentiality of selenium for male fertility was shown in the early 1980s (62–65). This conclusion was based on the results of a range of different experiments with mammals showing the following:

- In mild deficiency, Se is preferentially retained in rat testes (62).
- Mammalian semen is considered to contain the highest selenium concentration of all other body tissues (66).
- In the human, a significant positive correlation in the selenium concentration was demonstrated between the different reproductive organs, with the testis having the highest concentrations of this element (67).
- Progressive selenium deficiency was associated with morphological alterations of spermatids and spermatozoa (63) with subsequent complete disappearance of mature germinal cells (68).
- In selenium-deficient mice, the proportion of abnormal sperm ranged from 6.8 to 49.6%, whereas in the control group it was only 4.0–15.0%. The most frequently occurring abnormalities in sperm shape were found in the sperm head. However there was also a tendency of increasing abnormalities in other spermatozoa regions, including neck, mid-piece, and tail (69).
- In human semen, selenium was found mainly (more than 85%) in the seminal plasma and sperm motility was maximal when semen Se levels were between 50 and 69 ng/mL (70).

There are species-specific differences in the Se level in semen. For example, the seminal plasma level of Se was lowest in the human and the stallion, higher in ram and boar, and highest in the bull (71). Selenocysteine is shown to be the main form of Se in rat sperm and selenocysteine and selenomethionine were found in ovine sperm (72).

It is generally accepted that Se participates in various physiological functions as an integral part of a range of selenoproteins. The selenoprotein family includes at least 20 eukaryotic proteins (73). Expression of individual eukaryotic selenoproteins is characterized by high tissue specificity, depends on Se availability, can be regulated by hormones, and if compromised contributes to various pathological conditions (73). Most of the selenoproteins contain a single selenocysteine residue per polypeptide chain (74). GSH-Px and thioredoxin reductase (TR) are the most abundant antioxidant Se-containing proteins in mammals (75). The best characterized among selenoproteins is the GSH-Px family. In mammals it includes five members. The first member of this family, the so-called classical GSH-Px, was described in 1973 (76,77). The second selenoperoxidase, the phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px), was discovered 9 years later (78) and characterized in 1985 (79). Next, plasma glutathione peroxidase (pGSH-Px) was described in 1987 (80,81). The fourth selenoperoxidase, gastrointestinal glutathione peroxidase (GI-GSH-Px), was characterized in 1993 (82; for review see 83). Recently, a fifth member of Se-dependent glutathione peroxidases, a specific sperm nuclei GSH-Px (sn-GSH-Px), was characterized (84,85). In particular, this selenoenzyme has been identified in rat testes (84) to be a 34-kDa selenoprotein. It was localized in the spermatid nuclei and found to comprise about 80% of total Se present. It was identified as specific to the sperm nuclei GSH-Px with similar properties to PH-GSH-Px (85). The authors

showed that it differs from PH-GSH-Px in its N-terminal sequence. In rats, sn-GSH-Px is highly expressed in the nuclei of the late spermatids, where it is the only selenoprotein present (85). In Se-depleted rats the concentration of sn-GSH-Px decreased to a third of the normal level and chromatin condensation was severely disturbed (85).

The various GSH-Px are characterized by different tissue specificity and are expressed from different genes (86,87). The major function of these peroxidases is considered to be the removal and detoxification of hydrogen peroxide and lipid hydroperoxides (54,86). Since hydrogen peroxide is considered an intracellular messenger (88) and redox regulation can play a basic role in the activation of key transcription factors (89,90), it has been suggested that regulation of the delicate regional redox balance is one of the main functions of glutathione peroxidases (87). In contrast, the main function of sn-GSH-Px is protamine thiol cross-linking during sperm maturation (85).

Glutathione peroxidases are found in all mammalian tissues in which oxidative processes occur (73). The major role of these enzymes includes the reduction of hydrogen peroxide and organic peroxides to water and the corresponding alcohol, respectively. This is an important step in preventing production of reactive oxygen radicals. In general, the cytoplasmic GSH-Px is considered an "emergency enzyme" (73) responsible for prevention of detrimental effects of oxidative stress.

Maintenance of cellular redox state is another important function of the GSH-Px enzymes and GSH-Px forms are involved in such physiological events as differentiation, signal transduction, and regulation of pro-inflammatory cytokine production (91). Peroxynitrite scavenging by GSH-Px (92) could also play a prominent role in cell signal transduction events. Participation of GSH-Px enzymes in regulating biosynthesis of leukotrienes, thromboxanes, and prostaglandins is responsible for the modulation of inflammatory reactions, whereas PH-GSH-Px can bring about cytokine-induced transcriptional gene activation (for review see 73).

In general, different forms of GSH-Px perform their protective functions in concert, with each providing antioxidant protection at different sites of the body. For example, GI-GSH-Px could be considered to be a barrier against hydroperoxide resorption (87). Furthermore, in the gastrointestinal tract there are at least three more selenoproteins including plasma GSH-Px, selenoprotein P, and thioredoxin reductase (93). Plasma GSH-Px is an important antioxidant in plasma, which together with selenoprotein P and other antioxidant compounds maintain antioxidant protection. On the other hand, PH-GSH-Px is an important antioxidant inside biological membranes, where lipid peroxidation occurs and lipid hydroperoxides are produced.

GSH-Px activity ultimately depends on Se provision in the diet. However, some forms of GSH-Px Se are only synthesized when the Se supply is optimal. There are substantial differences among different forms of GSH-Px with regard to response to Se deficiency (87). The selenoproteins retained in tissues for longer periods during progressive Se deficiency are considered to have higher physiological significance in

comparison to those whose activities rapidly decline. In this respect, the main GSH-Px forms rank as follows (87):

GI-GSH-Px > PH-GSH-Px > Plasma GSH-Px = Cytosolic GSH-Px.

There is also a range of other selenoproteins identified, but their functions are less obvious (94,95). In relation to the antioxidant defense in avian sperm provided by GSH-Px, two other selenoproteins are of great importance: thioredoxin reductase (TR) and sperm capsular selenoprotein. Recently it has been appreciated that the redox status of the cell is a major determinant of many different pathways, including gene regulation (96). A thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase (97,98) and a thioredoxin system (thioredoxin/thioredoxin peroxidase/thioredoxin reductase) are believed to be the major players in this regulation (99,100). Together they supply electrons for deoxyribonucleotide formation, antioxidant defense, and redox regulation of signal transduction, transcription, cell growth, and apoptosis (101,102). Interestingly, TR can reduce not only thioredoxin but also oxidized glutathione (103). Therefore, these two systems are linked much more closely than previously considered. Experiments with yeast mutants lacking both the mitochondrial thioredoxin system and the mitochondrial peroxyredoxin system suggest an important role for thioredoxin, TR, and peroxyredoxin in the protection against oxidative stress (104).

It is interesting that sperm-specific thioredoxin (Sptrx) was recently identified and characterized in humans (105). The authors showed Sptrx mRNA to be expressed only in round and elongating spermatids, whereas the Sptrx protein is located in the cytoplasmic droplets of ejaculated sperm, suggesting that it might be an important factor in regulating critical steps of human spermatogenesis. Furthermore, a second member of this family, called Sptrx-2 and specifically expressed in human sperm cells, has been identified and characterized (106) and is considered to be a novel component of the human sperm axonemal organization. It seems likely that it is just the beginning of a deeper understanding of roles of new proteins in sperm function. For example, mouse Sptrx-1 has been cloned and characterized (107) as being similar to that described for the human and showing protein disulphide reducing activity in an enzymatic assay coupled to NADPH and TR. Therefore, it is just a matter of time before TR in the sperm is characterized and its essential role in sperm antioxidant protection is shown.

Sperm capsule selenoprotein (SCS) is localized in the mid-piece of the spermatozoa, where it stabilizes the integrity of the sperm flagella (108) and recently has been identified as PH-GSH-Px (109). However, recently it has been shown that the pertinent genes of rats and mice did not contain any TGA codons within the translated regions and, as a result, the essentiality of SCS for sperm function was questioned (73).

Unfortunately there are no data available concerning Se content in avian semen. However, GSH-Px was found to be expressed in chicken seminal plasma and sperma-

tozoa (Fig. 15.10). There are species-specific differences in activity and distribution of GSH-Px in avian semen. For example, on the one hand, in seminal plasma total GSH-Px activity was the highest in turkey and lowest in duck and goose (Fig. 15.11). In spermatozoa, on the other hand, the highest GSH-Px activities were found for goose and duck, and much lower GSH-Px activity was recorded for guinea fowl, turkey, or chicken (Fig. 15.12). Recently, it was shown that despite a high proportion of PUFA

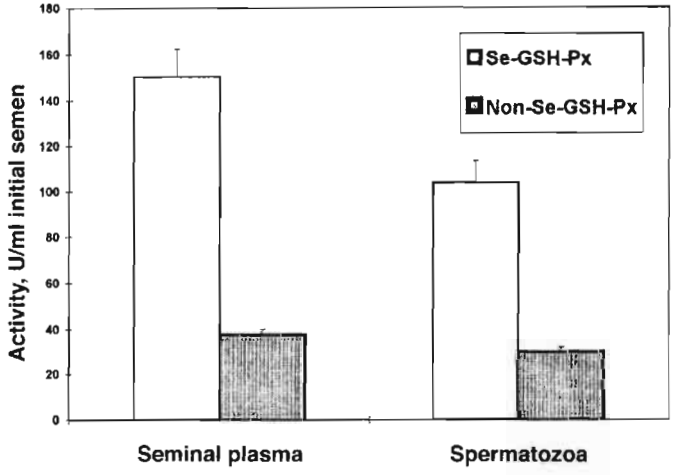

Fig. 15.10. Distribution of GSH-Px in chicken spermatozoa. Adapted from Surai *et al.*, 1998.

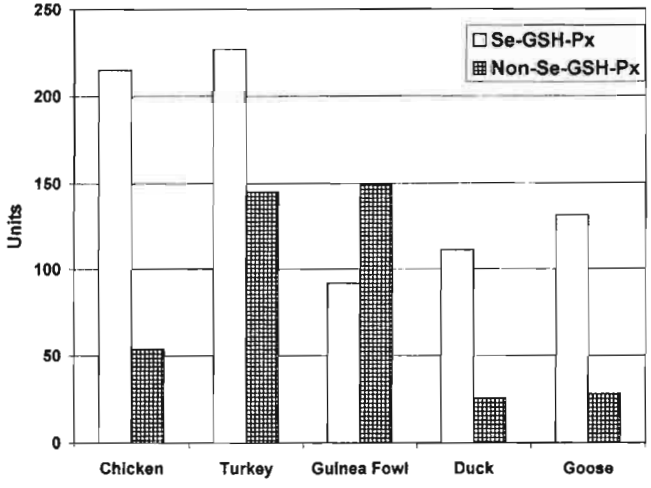

Fig. 15.11. GSH-Px activity in avian seminal plasma. Adapted from Surai *et al.*, 1998a.

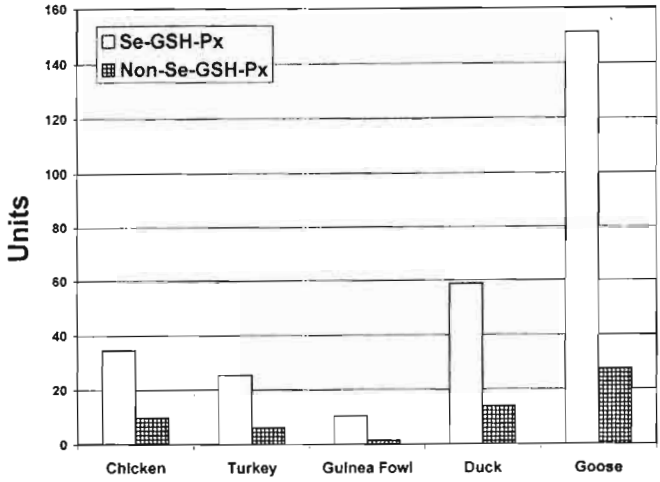

Fig. 15.12. GSH-Px activity in avian spermatozoa. Adapted from Surai *et al.*, 1998a.

and a low level of vitamin E, duck spermatozoa have the same susceptibility to lipid peroxidation as chicken spermatozoa (38). It has been suggested that an increased activity of Se-GSH-Px in duck semen compensates for the relatively low concentrations of other antioxidants.

It seems likely that GSH-Px is a universal antioxidant for spermatozoa because lipid hydroperoxides are extremely toxic for this kind of cell. For example, GSH-Px activity was found to be expressed in the semen of several mammalian species, including ram, dog, human, goat, and bull (110–112). However, there are species-specific differences in expression of this enzyme in semen. In bulls, for example, GSH-Px is exclusively associated with the seminal plasma and not found in spermatozoa (113,114). In contrast, GSH-Px activity in seminal plasma was low in man and ram and absent in boar and stallion (71). It has been shown that approximately two thirds of GSH-Px activity in bull semen was non-Se-GSH-Px (115). In the same experiment it was found that the MDA level was negatively correlated with Se-GSH-Px activity, and it has been suggested that Se-GSH-Px plays a role in protecting the acrosome membranes against disruption.

If selenium is limited in the diet (which is the case in many countries in the world), then dietary supplementation of this trace element should have a beneficial effect on the antioxidant defense in various tissues, including sperm. This was confirmed in our studies. Inclusion of Se in the diet of male chickens significantly increased Se-GSH-Px activity in the liver, testes, spermatozoa, and seminal plasma (Fig. 15.13 and 15.14; 116). As a result, a significant decrease in the sperm and tissue susceptibility to lipid peroxidation was observed. This protective effect was more expressed in stored semen as compared to fresh. In this respect, it is extremely important that an inducible form of the enzyme (Se-GSH-Px) represents more than 75% of

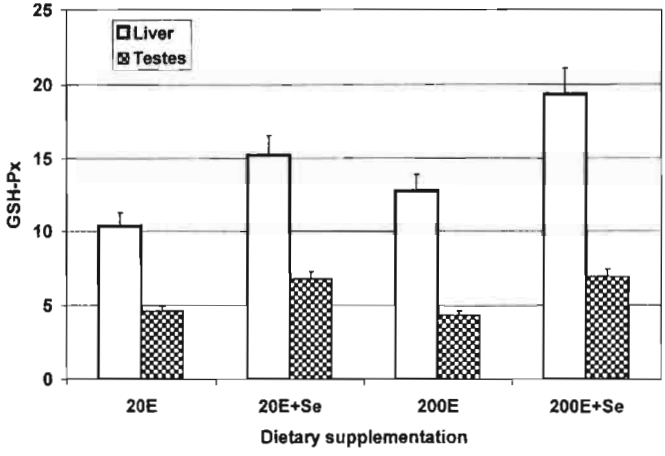

Fig. 15.13. Effect of vitamin E and vitamin E+ selenium on GSH-Px activity in chicken liver and testes. Adapted from Surai *et al.*, 1998b.

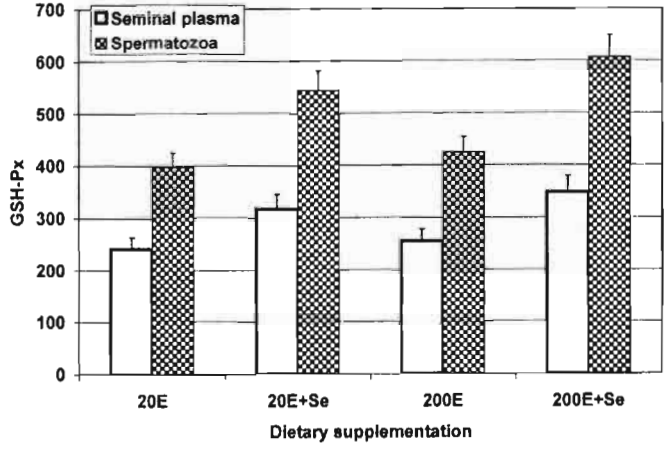

Fig. 15.14. Effect of vitamin E and vitamin E+ selenium on GSH-Px activity in chicken semen. Adapted from Surai *et al.*, 1998b.

the total enzymatic activity in chicken spermatozoa and more than 60% in the testes and liver of cockerels. 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 (117).

Unfortunately, data on the effect of Se supplementation of avian males on functional characteristics of spermatozoa and their fertilizing ability are very limited. Recently, Edens (118) showed that, when cockerels were fed on a basal diet contain-

TABLE 15.1
Spermatozoal Abnormalities (%) in Semen from Cockerels Fed on a Basal Diet or Diets Supplemented with Either Sodium Selenite or Selenomethionine[a]

Sperm morphology	Basal diet	Selenite	Selenomethionine
Normal sperm	57.9	89.4	98.7
Bent midpiece	18.7	6.2	0.7
Swollen midpiece	1.6	0.4	0.1
Ruptured midpiece	0.9	0.1	0.0
Swollen head	1.3	0.2	0.2
Corkscrew head	15.4	1.8	0.2
Coiled	3.2	0.8	0.0
Fragment/other	1.0	1.1	0.1

[a]Adapted from Edens (118).

ing 0.28 ppm Se without additional dietary supplementation of this trace element, the percentage of normal spermatozoa was only 57.9% (Table 15.1), and two major abnormalities seen were bent mid-piece (18.7%) and corkscrew head (15.4%). When this diet was supplemented with an additional 0.2 ppm Se in the form of selenite, the percentage of normal spermatozoa increased to 89.4% and abnormalities in the form of bent mid-piece and corkscrew head were decreased to 6.2 and 1.8%, respectively. However, when organic selenium was included in the cockerel's diet in the same amount, semen quality was further improved and those abnormalities decreased to 0.7 and 0.2%, and the percentage of normal spermatozoa increased to 98.7%. These results clearly showed that the form of dietary Se supplementation is a crucial factor of its efficiency, with organic selenium being much more effective than selenite. We suggested an explanation for this difference (119) based on an evolutionary approach because in nature birds will have only one form of selenium in their diet, organic selenium, mainly as selenomethionine, which is an integral part of any food item. Therefore, it seems that the digestive system of birds became adapted to this form of selenium, and as a result, there is a principal difference between organic and inorganic selenium in terms of their assimilation from the diet and use in the body with organic selenium being more effective (1).

Additional data from Edens (118) indicated that selenomethionine (0.3 ppm from 21 weeks of age) in the diet of Hubbard roosters improved semen quality to a greater extent than achieved by selenite at the same dose (Table 15.2). The sperm quality index significantly increased as well as the percentage of normal spermatozoa. At the same time, the proportions of various abnormalities in semen decreased. There was also a positive effect on fertility, which improved 0.56–1.03% by selenomethionine dietary supplementation. These experimental results confirmed the importance of selenium in maintaining chicken semen quality and specifically showed the advantages of organic selenium.

Similar positive responses of dietary Se supplementation have been seen with mammals, including cattle and humans (120–124), where Se supplementation enhances the *in vitro* motility and oxygen uptake of sperm.

TABLE 15.2
Effects of Sodium Selenite or Selenomethionine of Productive Parameters of Hubbard Breeder Hens[a]

	Farm 1		Farm 2	
Variable	Selenite	SeMet	Selenite	SeMet
Egg production, %	82.87	85.46	84.22	84.74
Fertility, %	96.12	96.68	96.23	97.26
Daily settable eggs	8799	9111	8379	8647
Hatchability, %	83.10	84.40	82.40	82.64

[a]Adapted from Edens (118).

Second Level of Antioxidant Defense—Vitamin E

Vitamin E is considered as the main antioxidant of biological membranes (125). 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, comprising less that 1 mol per 1000 mol of phospholipid (126). Nevertheless, due to its location inside the membrane on the water/lipid interface, vitamin E is able to effectively scavenge free radicals. Recently, the idea of vitamin E recycling from its oxidized form has received substantial attention. Vitamin E is considered to be recycled from its oxidized radical form by means of ascorbate, glutathione, cysteine, ubiquinols, lipoic acid, estrogens, carotenoids, and some other reductants. Therefore, the antioxidant protection in the cell depends not only on the vitamin E concentration and location but also relies on effective recycling. Indeed, if the recycling is effective then even low vitamin E concentrations are able to maintain high antioxidant protection in physiological conditions. One such example could be chicken embryonic brain (127). In this tissue, products of lipid peroxidation are almost undetectable in physiological conditions despite the very low vitamin E concentration. However, whether tocopherol recycling takes place in semen is not clear and this topic awaits investigation.

Vitamin E was first detected in turkey semen in 1981, and it has been shown that most α-tocopherol is located in the cells and only a very low concentration of this vitamin was detected in the seminal plasma (128). A similar distribution of vitamin E was shown for chicken semen with about 88% of the semen's vitamin E located in the spermatozoa (40). In general, depending on dietary supplementation, vitamin E concentration in chicken semen varied from 0.46 μg/mL (without vitamin E supplementation) up to 1.04–1.20 μg/mL (vitamin E dietary supplementation at level of 200 mg/kg) (45,116). It seems likely that vitamin E concentration in semen as well as in other tissues depends on the fatty acid profile of the diet. For example, when maize oil (5%) was included in the cockerel diet supplemented with 40 mg/kg vitamin E, the concentration of α-tocopherol in the semen was 1.1 μg/mL (129). However, tuna oil dietary supplementation decreased vitamin E concentration in the semen by more than

30% (129). α-Tocopherol is considered to be the main vitamin E form in spermatozoa, since the proportion of γ-tocopherol found in the spermatozoa was only 5–7% of total vitamin E (45). It is interesting that there are species-specific differences in vitamin E concentration in avian semen. For example, in duck spermatozoa vitamin E concentration was almost 3 times lower compared to chickens (38). In duck semen, most vitamin E was also located in spermatozoa. It is not clear at present if the vitamin E concentration in semen is associated with the fatty acid profile of the spermatozoa. When vitamin E is incorporated into sperm membranes, a high number of double bonds in the lipid fraction would increase the membrane's capacity to accommodate vitamin E.

The associations between sperm vitamin E content and spermatozoan physiological and biochemical characteristics have so far received only limited attention. An increased vitamin E concentration in turkey spermatozoa was associated with improved motility, viability, and fertilizing ability after artificial insemination (33,130). An increased vitamin E concentration in chicken semen was associated with improved spermatozoa progressive motility (131). In contrast, increased vitamin E supplementation of the pheasant diet was associated with decreased fertility, although the fertilizing persistence was longer with vitamin E supplementation (132).

Vitamin E concentration in spermatozoa is a reflection of its dietary supplementation. For example, inclusion of increased vitamin E doses in the turkey diet was shown to increase α-tocopherol concentration in spermatozoa (33,133). It has been suggested that vitamin E could be considered as a natural stabilizer of spermatozoa membranes. To test this hypothesis two main approaches were used. First, the release of glutamic-oxalacetic transaminase (GOT) from spermatozoa was used as a marker of sperm membrane integrity. We showed that during sperm storage *in vitro* the GOT activity increased in the medium and decreased in the spermatozoa (134). Similar changes in GOT activity were also observed in chicken spermatozoa during a freeze-thaw procedure (135), and a highly significant ($r = 0.99$) correlation was found between GOT activity in seminal plasma and the percentage of dead spermatozoa (136). The second approach was based on the inclusion of low concentrations of detergent (Triton X-100) in the sperm storage medium to induce sperm membrane damage. This treatment significantly increased the release of GOT from spermatozoa (137).

Therefore, the results of our experiments have shown the following:

- Turkey spermatozoa enriched with vitamin E released less GOT into the medium during sperm storage compared to the control group (138).
- During sperm cryopreservation a protective effect of increased vitamin E concentration in the spermatozoa was observed (139).
- An increased level of vitamin E in the turkey spermatozoa was associated with a reduction in susceptibility to Fe^{2+}-induced lipid peroxidation (34). This effect of vitamin E was confirmed with chicken semen (45).
- For inhibition of lipid peroxidation in the turkey spermatozoa, the efficiency of vitamin E, incorporated into the sperm membranes as a result

of its increased level in the diet, was almost 500 times higher than α-tocopherol inclusion in the diluent (128). Similar results were seen with chicken semen (129).

- Prevention of lipid peroxidation could be an important mechanism of the stabilizing effect of vitamin E on sperm membranes.

Thus it has been proved that vitamin E plays a role as a biological stabilizer of the sperm plasma membrane (1,33,50,140,141). Increasing the α-tocopherol content of the membrane was shown to make spermatozoa more resistant to the "unnatural" stresses incurred during artificial insemination, short-term storage, and cryopreservation (133,142). Increased dietary vitamin E supplementation of the goose (from 5 to 40 mg/kg diet) was associated with increased fertilizing ability of spermatozoa when used for artificial insemination (143).

It has been shown that inclusion of vitamin E in the chicken diet at the level of 200 mg/kg was associated with an almost twofold increase of α-tocopherol concentrations in whole semen, spermatozoa, and seminal plasma compared with that at 20 mg/kg supplementation (Fig. 15.15). However, vitamin E incorporation into spermatozoa membranes has a limit, since further increase in dietary vitamin E, from 200 to 1000 mg/kg, did not change α-tocopherol concentration in the semen (Fig. 15.16). As mentioned above, this could be a reflection of the limiting ability of the sperm membranes to incorporate vitamin E.

The dietary-induced increase in the α-tocopherol content of semen did result in a significant reduction in the susceptibility of the 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

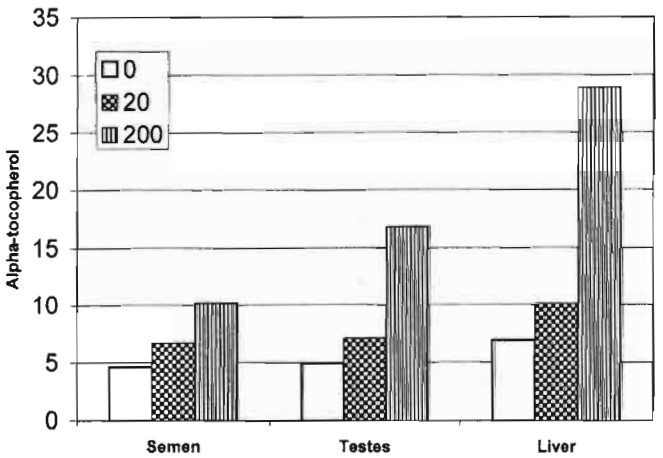

Fig. 15.15. Effect of dietary vitamin E on alpha-tocopherol concentration in semen (μg/mL), testes and liver (μg/g) of cockerels. Adapted from Surai *et al.*, 1997.

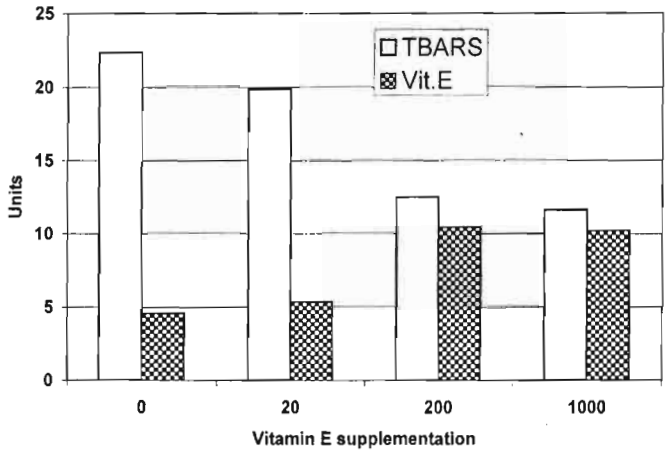

Fig. 15.16. Effect of dietary vitamin E on lipid peroxidation in chicken semen. Adapted from Surai *et al.*, 1997.

with α-tocopherol (116). During storage, the susceptibility of spermatozoa to lipid peroxidation significantly increased, probably due to initiation of spontaneous lipid peroxidation (35). In such conditions, the protective effect of vitamin E enrichment of the spermatozoa has been clearly demonstrated (116). Thus even the relatively limited enhancement of semen α-tocopherol content that was achieved by dietary means was found to produce significant benefits by reducing the susceptibility of the semen to lipid peroxidation.

Positive effects of increased vitamin E supplementation were recorded in mammalian species as well, including rams (144,145), boars (14), rats (146,147), bulls (148), and men (149–151).

Ascorbic Acid

Vitamin C (ascorbic acid, AA) acts as a potent water-soluble antioxidant in biological fluids by scavenging biologically relevant ROS and reactive nitrogen species (RNS; 152). In fact, AA is considered to be the most effective aqueous-phase antioxidant in human blood plasma (153). In cytosol, AA also acts as a primary antioxidant scavenging ROS and RNS (154). An indirect antioxidant role of AA is associated with the recycling of other antioxidants. In addition to vitamin E recycling, AA can regenerate such antioxidants as GSH, urate, and β-carotene from their respective radical species (152). A particularly important function of AA and GSH is their ability to neutralize hydroxyl radicals, since there are no enzymatic systems to scavenge it (155).

AA concentration in chicken semen was shown to be 210.2 ± 16.4 μM (40). On a molar basis, this concentration was more than 2.5 times higher than glutathione and more than 100-fold higher than α-tocopherol. AA was almost equally distributed

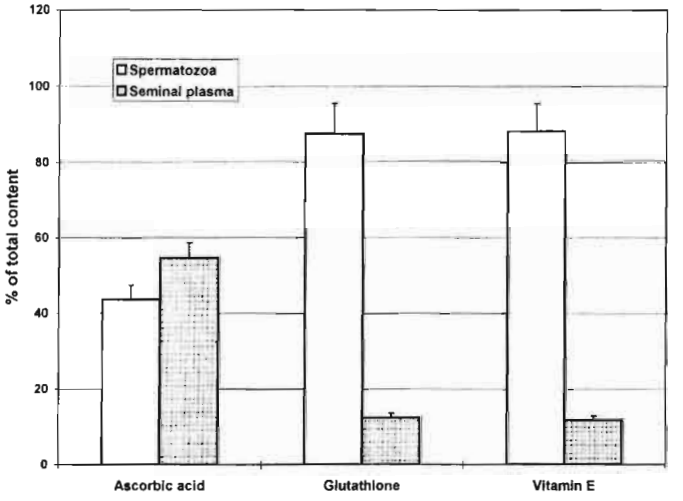

Fig. 15.17. Antioxidant distribution in chicken semen. Adapted from Surai *et al.*, 1998.

between the spermatozoa and seminal plasma (Fig. 15.17). These data suggest that AA plays an important role as a water-soluble antioxidant in avian seminal plasma.

Effects of AA on the animal reproductive system have recently been reviewed by Luck *et al.* (156) and main points of that review are as follows:

- There are direct effects of ascorbate deficiency on male fertility in laboratory and farm species, including bulls, guinea pigs, and rabbits.
- Ascorbate affected both the integrity of the tubular structure and the functionality of sperm.
- Ascorbate deficiency is associated with low sperm count, increased numbers of abnormal spermatozoa, reduced motility, and agglutination.

In human seminal plasma, ascorbate contributes to antioxidant protection almost twice as much as urate, and thiol levels are about one third of ascorbate (157). It is interesting that AA is accumulated in human and rat seminal plasma 5–10-fold compared with the serum level (158,159). In the seminal plasma of infertile men the AA concentration was found to vary widely, in a range of 93–954 μmol/L (160). Ascorbate levels in seminal plasma of asthenozoospermic individuals exhibiting ROS activity are significantly reduced (157). AA concentration in human seminal plasma was shown to be 612 μM, was negatively correlated with ROS production, and was positively correlated with the percentage of spermatozoa with normal morphology (161). AA also protects against endogenous oxidative DNA damage in human sperm (162). In bulls, the sperm motility correlated with the ascorbate concentration (163).

In cockerels housed under hot and humid tropical conditions, semen volume, motile sperm per ejaculate, and sperm number per ejaculate were significantly increased by AA supplementation at a level of 500 mg/kg (164).

Glutathione

Glutathione (GSH) is the most abundant nonprotein thiol in avian and mammalian cells and is considered to be an active antioxidant in biological systems, providing cells with their reducing milieu (165). Cellular GSH plays a key role in many biological processes (166):

- the synthesis of DNA and proteins
- cell growth and proliferation
- regulation of programmed cell death
- immune regulation
- the transport of amino acids
- xenobiotic metabolism
- redox-sensitive signal transduction

Furthermore, GSH thiolic group can react directly with (28, 167) the following:

- H_2O_2
- superoxide anion
- hydroxyl radicals
- alkoxyl radicals
- hydroperoxides

Therefore, a crucial role for GSH is as free radical scavenger, particularly effective against the hydroxyl radical (168). Usually, a low GSH concentration in tissues is associated with increased lipid peroxidation (169). Furthermore, in stress conditions, GSH prevents the loss of protein thiols and vitamin E (170) and plays an important role as a key modulator of cell signaling (171). Birds are able to synthesize glutathione.

GSH concentration in chicken semen has been shown to be 83.7 ± 9.12 μM (40), equivalent to about 2.34 nmol/10^8 spermatozoa. Similarly, in the rat, GSH concentration was reported to be 3.5 nmol/10^8 spermatozoa (172). These data are consistent with those for spermatozoa of goat, rabbit, ram, dog, boar, and human (110). Recently, in human spermatozoa, GSH concentrations were reported to be 3.49 nmol/10^8 spermatozoa (173). GSH concentration in bull spermatozoa was also similar: 3.1 nmol/108 spermatozoa (115). GSH was shown to play an important role in maintaining sperm motility and metabolism in experimental conditions (174). Adding GSH to the incubation medium had a preserving effect on equine sperm motility at the end of a 30-min incubation in the presence of a free radical-generating system (23). In an *in vitro* experiment, inclusion of GSH into the incubation medium caused a 57% decrease in lipid peroxidation in boar spermatozoa (14). GSH can also protect isolated rat spermatids from damage due to exposure to peroxidizing agents (175) and have a protective effect on rates of acrosome reaction and loss of motility over 24 h in human spermatozoa prepared by centrifugation (176). GSH was also effective in preventing the impairment of sperm motility observed in the presence of activated polymorphonuclea leukocytes (177). In contrast, addition of GSH to human sperm preparation medium had no significant effect on sperm progressive motility or baseline DNA integrity (178).

Metabolic Aspects of Antioxidant Defense

It is widely accepted that superoxide radical formation is usually the result of electron leakage from the mitochondrial electron transport chain due to uncoupled oxidative phosphorylation (55). There are also observations that leukocyte contamination of the semen is responsible for increased generation of free radicals. However, if semen contamination is minimal, metabolic differences between the species studied, especially in terms of mitochondrial oxidative phosphorylation activity (55), would determine differences in the rate of formation of superoxide radicals. Probably stress factors, responsible for uncoupling of oxidation and phosphorylation in mitochondria, could stimulate electron leakage and superoxide radical formation.

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 very dependent on oxidative metabolism to maintain optimal ATP levels (179). In this respect, the lower unsaturation of turkey sperm lipid could be an advantage in terms of prevention of lipid peroxidation. However, activities of antioxidant enzymes in turkey spermatozoa are also lower compared to chickens (61). Unfortunately, there are no data available on metabolic comparisons between other avian species.

Whereas the ratio of polyunsaturated fatty acids to antioxidants in the spermatozoa is a very important determinant of their survival *in vitro*, this could also be a factor in survival of spermatozoa in the oviduct. A unique feature of avian reproduction is spermatozoa storage within oviducal sperm storage tubules (SST; 180) for several weeks. Therefore, maintenance of membrane stability and prevention of lipid peroxidation during this high temperature (41°C) sperm storage could be an important strategy for avian species. In this respect the level of lipid unsaturation and fatty acid profile of the avian spermatozoa are different from those of mammals. Furthermore, turkey spermatozoa characterized by the lowest degree of lipid unsaturation have the longest fertile period in the SST. Therefore, an antioxidant role for the SST has been proposed (61).

There are also other unanswered questions in relation to species-specific features of fatty acid profile and antioxidant protection of avian spermatozoa. For example, duck spermatozoa containing the highest proportions of PUFA and characterized by the highest peroxidability index of their lipids, had a much lower vitamin E concentration compared to chickens (38). However the duck spermatozoa's susceptibility to peroxidation was similar to that of chicken spermatozoa (38), emphasizing an important role for the high activities of antioxidant enzymes in duck spermatozoa (61).

Therefore, species-specific features in carbohydrate metabolism and rate of mitochondrial oxidative phosphorylation are additional factors affecting free radical production in the semen. For example, our previous work (130) showed that chicken spermatozoa rely more on oxidative phosphorylation for energy production than waterfowl species. In fact, the lactate dehydrogenase (LDH) profile in spermatozoa of the chicken is opposite to that in duck or goose semen (Fig. 15.18 and 15.19). The

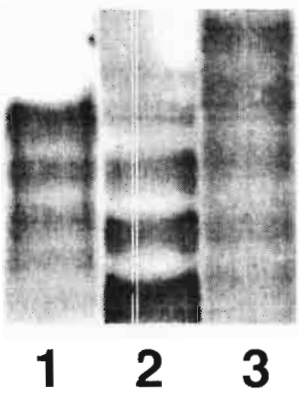

Fig. 15.18. Lactate dehydrogenase (LDH) profile in duck (1), chicken (2), and goose (3) spermatozoa. Adapted from Surai, 1991.

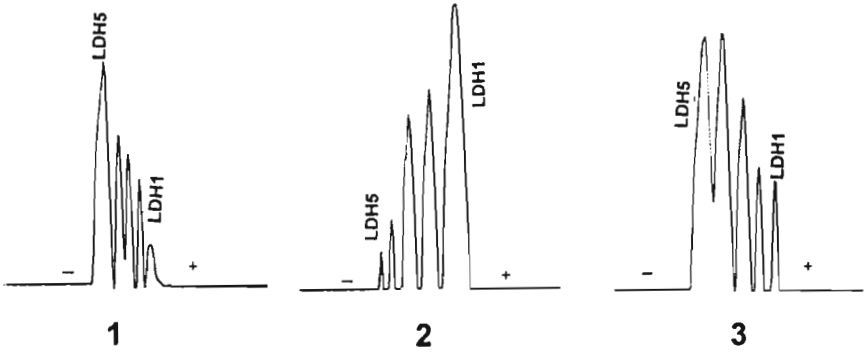

Fig. 15.19. Distribution of lactate dehydrogenase (LDH) isoenzymes in duck (1), chicken (2), and goose (3) spermatozoa. Adapted from Surai, 1991.

most active LDH isozymes in the chicken semen are LDH-1 and LDH-2, and the H-subunit of LDH comprises 81% of the total (Table 15.3), showing a clear preference for the Krebs Cycle and oxidative phosphorylation as a main route of energy production. It has been shown (181) that when cockerels with low cytochrome oxidase activity were removed from the flock, fertility was increased from 87.3 to 89.9%, confirming the importance of oxidative metabolism in maintenance of chicken semen quality.

In contrast, in duck and goose semen the main forms of LDH are LDH-5 and LDH-4, showing a preference for glycolytic energy production. This means that energy production in duck and goose semen is less dependent on oxidative phosphorylation, and so less free radicals may be generated in the mitochondria. It is interesting to

TABLE 15.3
Lactate Dehydrogenase (LDH) Isoenzyme Distribution on Avian Semen[a]

Species	LDH1, %	LDH2, %	LDH3, %	LDH4, %	LDH5, %	H-subunits, %
Chicken	53.14	23.52	17.77	4.53	1.05	80.8
Goose	3.92	7.34	19.74	30.67	38.34	26.95
Duck	4.89	8.82	13.46	18.65	54.13	22.93

[a]Adapted from (130) and (181)

note that the LDH profile in testes of cockerels and duck and goose (Fig. 15.20) is very similar to that observed in spermatozoa, with LDH1 and LDH2 being the major isoenzymes in cockerel's testes. However, LDH profile in breast muscle (mainly LDH5) or heart (mainly LDH1) of goose and chicken are very similar. Therefore the specific differences in LDH profile in testes and spermatozoa between chicken and waterfowl males need further investigation.

Since copulation in waterfowl could be conducted on the water surface, it could well be that protective mechanisms exist to prevent any water appearance in the reproductive tract and, therefore, the oxygen supply there could be lower in comparison to chicken. Furthermore, in the chicken and turkey, natural mating consists of semen deposition in the lower (abovarian) vagina due to the presence of a vestigial penis, which, in these species, prevents any vaginal penetration of the female at copulation

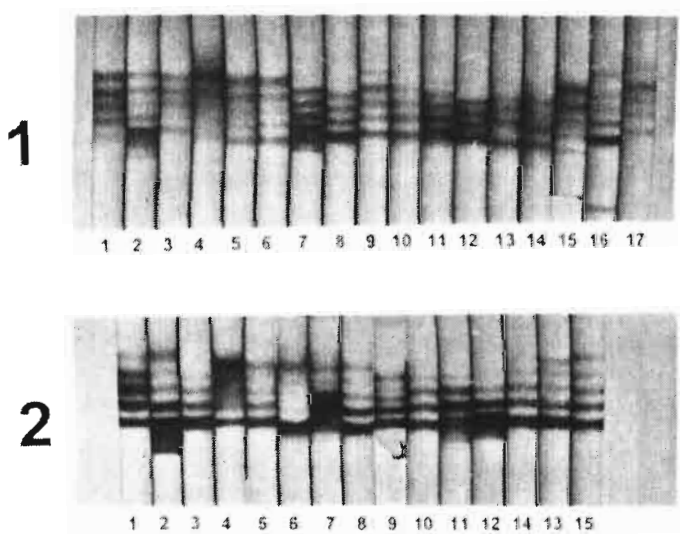

Fig. 15.20. Lactate dehydrogenase (LDH) profile (gel-electrophoresis) in various tissues of goose (1) and chicken (2) males. 1, liver; 2, heart; 3, testes; 4, breast muscle; 5, thigh muscle; 6, gizzard; 7, kidney; 8, lung; 9, spleen; 10, skin; 11, brain; 12, pancreas; 13, thyroid; 14, adrenals; 15, thymus; 16, blood hemolysate; 17, blood plasma.

(182). Spermatozoa in these species must therefore migrate through most of the luminal portion of the vagina before reaching the storage sites, a migration performed in the somewhat oxygenated environment of the vaginal mucosa. By contrast, in ducks the existence in the female of a nontubular vagina (presence of a double "S-like" portion) located approximately at mid-vagina, and in the male of a well-developed penis, suggest that ejaculated semen is deposited in the upper vagina (182), which, due to its anatomical specificities, is likely to be isolated from a direct contact with ambient air. Therefore, it has been suggested (38) that the antioxidant defense of duck spermatozoa is not, in the natural situation, as crucial as it is in the chicken and the turkey. However, increased activity of antioxidant enzymes in duck spermatozoa (38) could be another important factor on the antioxidant/pro-oxidant balances in semen.

In general there are three major features of avian semen that put it under pressure of oxidative stress:

- Limitations in antioxidant recycling. Because of very low activity or even absence of the hexose monophosphate shunt in avian spermatozoa (183), the production of NADPH, the coenzyme for glutathione reductase, is limited. This means that recycling in the chain vitamin E-vitamin C-GSH in the spermatozoa is limited as well. In such a situation, the primary defense preventing conversion of superoxide radical to more powerful radicals (for example, OH*) would be of great importance for spermatozoa survival.
- Sperm storage within oviductal sperm storage tubules (SST) at a body temperature of 41°C can be considered a risk factor for lipid peroxidation, and an antioxidant role of the SST has been proposed
- It is suggested that spermatozoa cannot carry out extensive biosynthetic repair of damage (51). Therefore, any damaging alteration to the membrane irreversibly alters sperm functions and the antioxidant protection is thus absolutely vital for maintaining the fertilizing ability of spermatozoa.

Conclusions

- Avian spermatozoa are rich in PUFA, which makes them vulnerable to lipid peroxidation, especially during *in vitro* manipulation. In particular, docosatetraenoic (22:4n-6) and arachidonic (20:4n-6) fatty acids are the most vulnerable to lipid peroxidation.
- Lipid peroxidation in semen is considered an important mechanism of impaired sperm quality and reduced fertilizing ability.
- Excessive free radical generation by spermatozoa could be induced by various factors, including the redox cycling of xenobiotics, excessive NADPH oxidase activity, the increased availability of transition metals, and impaired antioxidant protection due to dietary deficiencies, age, or genetic factors (27).

- Antioxidant systems of semen play an important role protecting spermatozoa membranes against the damaging effects of free radicals and toxic products of their metabolism.
- There are species-specific, age-related differences in the expression of antioxidant systems in avian semen.
- Sperm enrichment by DHA is shown to decrease antioxidant protection of the spermatozoa and cause lipid peroxidation. Dietary supplementation of increased vitamin E levels is found to be effective to prevent lipid peroxidation in such cases.
- Vitamin E is considered to be an effective membrane-stabilizing antioxidant of avian semen.
- An antioxidant function of SST has been suggested, and species-specific differences in strategy of antioxidant defense have been described.
- Enhancement of the antioxidant capacity of semen could present a major opportunity for improving male fertility.
- The antioxidant/pro-oxidant balance in avian semen is an important element in maintaining membrane integrity and functions, including sperm viability and fertilizing capacity. The antioxidant system can be suggested to be a crucial element of such a regulation.
- A regulating role of ROS in sperm capacitation and the acrosome reaction in mammalian species has been demonstrated; but their role for avian species 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.

Acknowledgments

We are grateful to the Scottish Executive Environment and Rural Affairs Department for financial support and to the WPSA for the Research Award to Peter F. Surai.

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