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Antioxidants and poultry semen quality

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Avian spermatozoa are characterised by high proportions of polyunsaturated fatty acids (PUFAs) which is associated with increased susceptibility to reactive oxygen species (ROS) and lipid peroxidation. Recent advances in avian reproduction have focused on the potential of ROS as one of the prime mediators of infertility. Although ROS are involved in many physiological functions of spermatozoa, their excessive production may result in oxidative stress. The production of ROS is enhanced during unfavourable environmental and stressful conditions, and an effective scavenging system is essential to counter the production of ROS. Antioxidants are compounds that suppress the formation of ROS. Enzymatic and non-enzymatic antioxidants build a powerful antioxidant system in avian semen which is able to protect it against ROS and lipid peroxidation. The balance between ROS and antioxidants in avian semen is a fundamental determinant of membrane integrity, sperm viability and fertilising ability. A regulating role of ROS in sperm function in mammalian sperm along with their counteractive mechanisms have been extensively studied; but their role for avian species reproduction remains to be elucidated. Various antioxidants belonging both to enzymatic and non-enzymatic groups can counter oxidative stress. Hence the rationale of using antioxidants is advocated.

Keywords: semen; avian; quality; oxidants; antioxidants

Introduction

Fertility is the first and most important requisite of poultry breeding. The number of fertile eggs produced for hatching dictates the ultimate profitability of hens. Infertility is a major economic factor in the poultry industry. Although males and females both contribute to decreasing fertility, low fertility is thought to be largely a problem in males. There are many causes of low fertility, however, it is well documented that age has an adverse effect on the reproductive success of male chickens (Zhang *et al.*, 1997; Lin *et al.*, 2005).

Lipids are among major components of sperm membranes involved in a series of processes, which ultimately influence their fertilising potential (Alvarez and Storey, 1995). In cockerels, lipids form an integral part of the sperm membrane and are

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involved in the various stages of maturation, capacitation and acrosome reaction, a series of biochemical and functional changes required before or as part of the process of fertilization (Koyanagi *et al.*, 1988). The high levels of PUFAs in avian spermatozoa render these cells vulnerable to deleterious effects of lipid peroxidation, (Fujihara and Howarth, 1978; Wishart, 1984), which is correlated to male fertility.

As a consequence of extensive lipid peroxidation, ROS are constantly being produced which are detrimental to the fertilising ability of sperm (Surai *et al.*, 2000; Sikka, 2001; Agarwal *et al.,* 2003). Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major ROS present in seminal plasma. Due to increased production of ROS, oxidative stress occurs which is the imbalance between pro-oxidants and antioxidants (Agarwal *et al.*, 2003; Urso and Clarkson 2003). Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of ROS (Castillo *et al.* 2001). However, when ROS generation exceeds the body's antioxidant production capacity, oxidative stress develops. Oxidative stress reduces gamete numbers, decreases sperm motility and increases percentage of dead cells (Alkan *et al.*, 1997; Surai *et al.*, 2000; Sikka, 2001; Agarwal *et al.*, 2003).

In an attempt to overcome fertility problems due to poor male performance, several studies have suggested that certain antioxidants could protect the spermatozoal membrane and increase spermatozoal viability. Variety of defence mechanisms encompassing antioxidant enzymes, biomolecules and vitamins are available which benefits from the risk of ROS and appear to be necessary for survival and functioning of spermatozoa. Therefore, the aim of present document is to review the effect of certain antioxidants on the quality of avian semen.

Physiology of ROS

Poor sperm quality has been linked to the excessive production of ROS as a result of lipid peroxidation in semen (Gomez *et al.*, 1998; Agarwal *et al.*, 2003). Depending upon the type, concentration, location and time of exposure, ROS may have beneficial or harmful effects (Agarwal and Saleh, 2002). Appropriate motility is not sufficient to ensure fertilization, as sperm must pass through a physiological process of capacitation in the female reproductive tract (Visconti and Kopf, 1998). Under physiological condition, spermatozoa produce small amount of ROS, such as superoxide anion, which effectively plays a role in capacitation and the acrosomal reaction (Agarwal *et al.*, 2003).

The high levels of polyunsaturated fatty acids in the plasma membrane and cytoplasm of spermatozoa makes it vulnerable to the attack of ROS (Alvarez and Storey, 1995). Hence, excessive production of ROS is one of the basic causes of infertility (Sharma and Agarwal, 1996; Alkan *et al.*, 1997). Gomez *et al.* (1998) reported that the amount of ROS produced by spermatozoa is inversely proportional to the quality of sperm in the original semen sample. Hydrogen peroxide is one of the major ROS produced by the spermatozoa which causes immobilization of spermatozoa via intracellular ATP and subsequent decline in the axonemal proteins (Kemal *et al.*, 2000; Misro *et al.*, 2004). High level of hydrogen peroxide results in the lipid peroxidation and ultimately cell death. Spermatozoa lose most of their cytoplasm while undergoing differentiation during their final stage of maturation. Under certain circumstances, the cytoplasm is transformed into cytoplasmic droplet. Such spermatozoa are thought to be immature, functionally defective and capable of producing high concentration of ROS (Sikka, 2001; Agarwal *et al.*, 2003).

Mitochondria contribute to the production of ROS through nicotinamide adenine

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dinucleotide (NADH)-dependent oxido-reductase systems (Hallak *et al.*, 2001). Mitochondria are present abundantly in spermatozoa, where they provide energy for motility. Production of ROS is significantly increased in damaged mitochondria, which in turn affect negatively mitochondrial function in spermatozoa (Evenson *et al.*, 1982). Inner mitochondrial membrane potential (MMP) is very important in the determination of sperm function. According to Wang *et al.* (2003), MMP is lower in the spermatozoa of infertile subjects with high level of ROS production and is positively correlated with sperm concentration.

Mitochondria play a key role in the process of apoptosis. High levels of ROS disrupt the outer and inner mitochondrial membrane, releasing cytochrome C protein and activating caspase enzymes to induce apoptosis (Wang *et al.*, 2003). Elevated level of cytochrome C in seminal plasma indicates significant mitochondrial damage by ROS. DNA damage and cross linking protein can also disturb semen quality (Sharma *et al.*, 1996). A spermatozoon with dysfunctional DNA is unable to fertilize an ovum. Therefore, the fertilization rate decreases with increasing DNA damage (Sun *et al.*, 1997).

Concept of antioxidants

The high proportions of PUFAs in the membranes of avian spermatozoa reflect the specific need of their membrane for high fluidity and flexibility which are essential for sperm mobility and fusion with the ova (Donoghue and Donoghue, 1997; Zaniboni *et al.* 2006). This functional advantage is, however, associated with disadvantage in terms of vulnerability of sperm to ROS and lipid peroxidation (Aitken *et al.*, 1989; Sikka *et al.*, 1995; Kelso *et al.*, 1996; Agarwal and Prakaran, 2005). This is why antioxidant protection is a vital element in maintaining membrane integrity, motility and fertilising ability (Donoghue and Donoghue, 1997).

The balance of ROS can be considered as the balance of 'creation and destruction'. Under normal physiological circumstances, there is an appropriate balance between ROS and antioxidants (Agarwal and Prakaran, 2005); increased production of ROS and decrease level of antioxidants result in oxidative stress. Consequently, imbalances between pro-oxidants and antioxidant concentration will have a negative impact on semen quality (Sikka *et al.*, 1995). This will result in increased lipid peroxidation, decreased sperm motility, viability and, ultimately, infertility.

Antioxidants, in general, are compounds and reactions that remove, scavenge or suppress the formation of ROS, or oppose their actions (Sikka *et al.*, 1995). A variety of biological and chemical antioxidants that attack ROS are under investigation. Among the well known biological antioxidants are superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) have been well documented in literature (Froman and Thurston, 1981; Lin *et al.*, 2005; Eid *et al.*, 2006). Within the category of chemical antioxidants, both natural and synthetic products have garnered attention due to their usefulness in reproduction and fertility management (Agarwal and Prakaran, 2005). It has been suggested that natural antioxidants (vitamin E, ascorbic acid) and enzymes (SOD and GSH-Px) create an integral system in avian semen, capable of protecting them against ROS and toxic products of metabolism (Froman and Thurston, 1981; Pardue and Thaxton, 1986; Donoghue and Donoghue, 1997; Lin *et al.*, 2005; Eid *et al.*, 2006).

The delicate balance between the production of ROS and antioxidants defence is considered to be an important determinant of semen quality particularly its fertilising ability (Sikka *et al.*, 1995). The relationship between ROS and antioxidant status in avian semen, as well as the possibility of modulation by nutritional means, are important considerations (Surai *et al.*, 2003). Antioxidants can be broadly categorised into two

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groups *i.e.*, enzymatic and non enzymatic. Their role in avian semen is elucidated below (Agarwal and Prakaran, 2005).

Vitamin E

One dietary antioxidant compound which has received considerable emphasis is vitamin E. Vitamin E was first detected in turkey semen in 1981 and later also in chicken semen. It was shown that 88% of chicken semen vitamin E is located in the spermatozoa. In general, depending upon diet supplementation, vitamin E concentration in chicken semen varied from 0.46 μg/ml (without vitamin E supplementation) up to 1.04 μg/ml (vitamin E dietary supplementation at level of 200 mg/kg) as reported by Surai *et al.* (1997). However, no further improvement was observed by increasing vitamin E concentration up to 1000 mg/kg of feed in the same experiment. Structurally, vitamin E has been categorised as α (alpha), β (beta) and γ (gamma) tocopherol. α-tocopherol is considered to be the major vitamin E in spermatozoa, followed by γ-tocopherol which is only 5-7% of total vitamin E (Surai *et al.*, 1997).

According to Lin *et al.* (2005) fertility and duration of fertility are the most important economics traits and for male breeder poultry, and they may be affected by environmental factors such as temperature and photoperiod as well as dietary intake of vitamin E (Eid *et al.*, 2006). Vitamin E effectively scavenges free radicals and reacts with them, producing stable ROOH groups. It has been suggested that vitamin E provides biological stability to the spermatozoal plasma membrane (Surai *et al.*, 2000). The dietary increase in the Vitamin E content of semen was found to result in a significant reduction in the susceptibility of the semen to lipid peroxidation (Niki, 1993; Lin *et al.*, 2005). An increased level of vitamin E in the chicken spermatozoa has been associated with a reduction in susceptibility to Fe^{+2}-induced lipid peroxidation (Surai *et al.*, 1997).

Dietary supplementation of vitamin E to maintain and enhance performance is routinely used in poultry production. The results obtained varied depending upon the level and duration of feeding diets supplemented with vitamin E, genetic stocks, age and measurement criteria (Siegel *et al.*, 2001). *In vitro* studies have also shown that vitamin E is a major chain-breaking antioxidant in the sperm membrane. Cryopreservation and thawing procedures are linked to a significant reduction in sperm motility induced by ROS and these effects can be effectively combated by addition of vitamin E to cryoprotectants (Surai *et al.,* 2000). Improvements in semen quality attributed to vitamin E supplementation have been associated with prevention of chain reaction oxidation (Niki, 1993) and inhibition of lipid peroxidation of sperm (Fujihara and Howarth, 1978).

Adequate dietary vitamin E is necessary not only in the diet of cockerels to enhance fertility, but also to ensure that sufficient vitamin E is passed onto the developing embryo after hatching. It has been suggested that diets should contain minimum of 10 mg vitamin E/kg feed (Whitehead and Portsmouth, 1989). Lin *et al.* (2005) demonstrated that, in Taiwan, native chicken cockerels consuming supplemental vitamin E at the rate of 40 to 160 mg/kg feed have improved sperm viability and motility and that long term omission of the vitamin may result in adverse effects. Lin *et al.* (2005) found that supplementing vitamin E at a rate of 80 and 160 mg/kg feed tended to increase plasma testosterone concentration and sperm concentration at 39 weeks of age.

Age-related decreases in the fertility of cockerels are associated with decreased levels of vitamin E in the testes (Surai *et al.*, 1997). Decreased concentration of functional polyunsaturated fatty acids ($C_{20\text{-}22}$) in phospholipids of spermatozoa can be minimised

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with vitamin E supplementation up to 200 mg/kg, but no further improvement was noticed when its concentration was increased to 1000 mg/kg. In the same study, TBARS (thiobarbituric acid reactive substances - an indicator of lipid peroxidation) was reduced by approximately 50% during the incubation of semen *in vitro* under pro-oxidant conditions by higher supplementation of vitamin E (1000 mg/kg of feed). Lin *et al.* (2005) found that lack of vitamin E over 39 weeks of age was associated with lower semen quality. Furthermore, fertility can be improved by supplementing 160 mg/kg vitamin E at 49 weeks of age (Lin *et al.*, 2005). In addition, supplementation of selenium enhances the role of vitamin E by increasing activity of GSH-Px, reducing susceptibility of spermatozoa to lipid peroxidation (Surai *et al.*, 1998).

Another possible route for inhibition of lipid peroxidation is via the direct inclusion of vitamin E into the semen diluents. A moderate concentration of 80 µg/ml vitamin E was found to improve the fertilising potential of turkey semen stored at 24°C for 24 hours. In the turkey, addition of vitamin E to the diluted semen resulted in the improved membrane integrity, better survivability along with higher motility and lower lipid peroxidation (Donoghue and Donoghue, 1997). However, complementary experiments proved that dietary supplementation of vitamin E was much more influential in inhibition of lipid peroxidation than direct integration into diluents (Surai *et al.*, 2000). The susceptibility of testes homogenates to *in vitro* peroxidation was also reduced considerably by dietary supplementation of vitamin E (Surai *et al.*, 1998).

Vitamin C

Vitamin C (ascorbic acid) is a water soluble antioxidant which is normally synthesised by the chicken. It has been recommended in poultry feed as supplementation to alleviate stress on the assumption that during stress the requirements may exceed the synthesizing ability (Gous and Morris, 2005). Physiological stressors like heat, diseases or overcrowding may augment the chicken requirement for ascorbic acid (Nockels, 1984). Chickens require vitamin C for amino acid and mineral metabolism, as well as for synthesis of hormones like testosterone (McDowell, 1989), which is essential for the reproductive performance of male birds. Vitamin C is involved in the production of white blood cells and thus enhances the immune status of the bird. Nowaczewski and Kontecka (2005) documented that ascorbic acid contributed 65% of the antioxidant capacity of the seminal plasma.

Within normal environmental conditions, dietary ascorbic acid has been reported to improve fertility in poultry. Pardue and Thaxton (1986) reported that supplementation of ascorbic acid (100 ppm/kg feed) resulted in increased testicular weight of 8 week old broiler breeder cockerels. Dobrescue (1987) demonstrated that supplementation of vitamin C at the rate of 150 ppm/kg feed increased semen volume, sperm concentration and total number of sperm produced in turkey breeder toms. Additionally, he found significantly decreased sperm agglutination. Harris *et al.* (1974) reported decreased sperm agglutination and improved fertility by supplementing ascorbic acid. Peebles and Brake (1985) concluded that feeding ascorbic acid to broiler breeder at the rate of 50 ppm/kg feed improved fertility compared to the control group. In contrast, McDaniel *et al.* (1998) reported that ascorbic acid (500 ppm/kg feed) did appear to improve semen quality and fertility. Elansary *et al.* (1999) reported increased ejaculated volume, sperm concentration and sperm motility in vitamin C fed cockerels compared to the control. Nowaczewski and Kontecka (2005) suggested 100 mg/kg vitamin C supplementation in feed for prophylactic use in the nutrition of breeding pheasants reared in aviaries.

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High ambient temperature has been found to adversely affect the semen characteristics of the male chickens. Blood ascorbic acid level is inversely proportional to environmental temperature within the range of 21-31°C. The exact mechanism of heat stress affecting fertility is not known. McDaniel *et al.* (1995) concluded that during heat stress period, the increase in body temperature has negative effect on gamete formation and the fertilisation process. Fewer sperm from heat stressed broiler breeder males are stored in the hen's oviduct as compared to those which are maintained in a thermoneutral environment (McDaniel *et al.*, 1996). The chicken may not have the capability to produce enough ascorbic acid to meet the physiological demand (Pardue and Thaxton, 1986). McDaniel *et al.* (1996) reported increased percentage of dead sperm in broiler breeder kept in heat stressed environment (32°C).

It has been shown that supplementation with 500 mg/kg of vitamin C in the diet of broiler breeders housed under hot and humid tropical conditions improved semen volume, the number of motile spermatozoa per ejaculate, and spermatozoal numbers per ejaculate (Monsi and Onitchi, 1991). Vitamin C, at rate of 200 mg/kg in feed, is also beneficial for reproductive aviary pheasants housed under heat stress (Nowaczewski and Kontecka, 2005). However, McDaniel *et al.* (2004) found that excessive administration (500 and 1000 ppm) of ascorbic acid resulted in depression of fertility over each day post-insemination when the males were heat stressed and concluded that only specific level of ascorbic acid are beneficial to stressed poultry.

Selenium

Selenium is an essential element that has an important role in animal reproduction (Leonhard, 2000; Surai *et al.*, 2000; Barber *et al.*, 2005; Dimitrove *et al.*, 2007). The effect of selenium as an antioxidant has been explained through its role as component of the antioxidant enzyme GSH-Px (Surai *et al.*, 1998; Eid *et al.*, 2006). There are different kinds of selenoproteins which participate in the regulation of various physiological functions including antioxidant protection and stability of sperm cell membrane. Recently it has been established that part of GSH-Px is converted from the enzymatic form to structural part of the sperm. The importance of selenium is evident from the fact that selenium-supplemented semen shows better characteristics of storage, sperm motility and the slow release of total lipids and phospholipids from spermatozoa to seminal plasma during storage (Dimitrove *et al.*, 2007). Further, in selenium deficient roosters, the number of sertoli and leydig cells decreased significantly which are required for spermatogenesis and testosterone production respectively (Edens and Sefton, 2002).

As the role of selenium in male poultry fertility has been recognised for many years, inclusion of selenium in the poultry diet has been emphasised. Many years of research has demonstrated that selenium deficiency causes poor male fertility in poultry chickens and impaired reproductive performance (Barber *et al.*, 2005). Selenoprotein contributes to the stability of spermatozoa, therefore when the deficiency of selenium occurs, the quality of semen declines (Leonhard, 2000). Surai *et al.* (2000) reported that GSH-Px is selenium dependant enzyme which is an essential component of the antioxidant system of avian semen. Under storage conditions, Dimitrove *et al.* (2007) found that inclusion of 0.3 ppm selenium in addition to 0.1 ppm in the turkey basal diet showed positive effect on lipid composition of semen and better semen integrity in the form of improved fertilising ability of spermatozoa. Barber *et al.* (2005) demonstrated that *in vitro*, addition of selenium to the broiler breeder semen at very high (7896 µg/l) dose may be detrimental to the sperm in terms of semen quality index. It explains that *in vivo* the

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mineral may indirectly improve the semen quality during spermatogenesis rather than acting directly on spermatozoa. This may also be related to the form of selenium used.

Selenium deficiency has been known to negatively affect the antioxidant defence mechanisms in chicken semen (Surai *et al.*, 1998). Edens and Sefton (2002) demonstrated that, when cockerels were fed with additional 0.2 ppm selenium to the basal diet containing 0.28 ppm selenium, the percentage of normal spermatozoa was increased and abnormalities in the form of bent mid-piece and corkscrew head were significantly decreased. The percentage of normal spermatozoa were further increased and the mid piece abnormality decreased by the addition of more 0.2 ppm organic selenium to the diet. It is clear that the mid-piece of spermatozoa of the Se-deficient male is dysfunctional, and can compromise sperm motility and fertilising capacity. Inclusion of selenium in the male diet also significantly increased Se-GHS-Px activity in spermatozoa and seminal plasma (Surai *et al.*, 1998), resulting in significant decreases in the sperm susceptibility to lipid peroxidation. This protective effect was more pronounced in stored semen as compared to fresh semen.

Carnitine

Carnitine is a vitamin-like-amino acid synthesised from methionine and lysine which acts as receptor inside cell for activated fatty acids (Adabi *et al.*, 2008). Carnitine has antioxidant properties that protect the sperm membrane against toxic ROS (Neuman *et al.*, 2002; Stradaioli *et al.*, 2004; Sarica *et al.*, 2007; Adabi *et al.*, 2008). High concentrations of carnitine are present both in seminal plasma and spermatozoa, where it reduces the availability of lipid for peroxidation by transporting polyunsaturated fatty acids into mitochondria for β-oxidation to generate energy for sperm motility (Neuman *et al.*, 2002; Zhai *et al.*, 2007). Carnitine has the ability to chelate with free iron, thus preventing the production of ROS (Neuman *et al.*, 2002) due to pro-oxidant iron activity. Carnitine can protect other antioxidants against potential peroxidative damage, *e.g.* it works together with SOD to protect lipid membrane surrounding sperm and plays a key role in reducing lipid peroxidation, protecting cells from peroxidation damage (Froman and Thurston, 1981; Stradaioli *et al.*, 2004).

Breeder birds and roosters supplemented with L-carnitine have shown improvements in semen traits and fertility parameters due to inhibiting lipid peroxidation of the sperm membrane (Neuman *et al.,* 2002; Sarica *et al.*, 2007; Adabi *et al.*, 2008). Dietary inclusion of 250 to 500 ppm L-carnitine in feed has been recommended to achieve significant improvements in sperm count and quality for roosters (Neuman *et al.*, 2002; Sarica *et al.*, 2007; Adabi *et al.*, 2008). Nuerman *et al.* (2002) demonstrated that feeding L-carnitine to broiler breeder increased sperm count, reduced lipid peroxidation and number of multinucleated giant cells. Likewise, Sarica *et al.* (2007) confirmed that inclusion of 250 mg/kg or 500 mg/kg to the basal diet of male Japanese quails significantly increased sperm viability and decreases multinucleated giant cells per testes. Adabi *et al.* (2008) concluded that L-carnitine, at the rate of 250 and 500 ppm feed, produced optimum results in the form of semen volume, sperm motility and live sperm count, with no significant effects on abnormal sperm percentage in African black neck ostrich. Zhai *et al.* (2007) demonstrated in White Leghorn male birds that long term consumption of 125 ppm L-carnitine beginning at hatch resulted in persistent increase in sperm concentration and suggested that L-carnitine's antioxidant influence on sperm production begins before the onset of puberty.

Plausible roles for carnitine as an antioxidant can be explained by the manner in which it improves pyruvate utilisation, an elective energetic substratum for sperm motility.

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Since the correct ratio of acetyl CoA/CoA ratio is essential in order to maintain the normal function of Kreb's cycle for sufficient production of adenosine triphosphate (ATP). High concentration of acetyl CoA inhibits pyurvic dehydrogenase enzyme activity, consequently metabolic flow of pyruvate into the Kreb's cycle slows down. Carnitine is transferred into acetylecarnitine through carnitine acyl transferase (CAT) which reduces acetyl CoA/CoA ratio with increased production of ATP, preserving high motility of spermatozoa (Neuman *et al.*, 2002; Stradaioli *et al.*, 2004; Adabi *et al.*, 2008). Moreover, it is well known that stabilisation of sperm head and tail takes place in the epididymis through the formation of intra and inter-molecular disulfides (Adabi *et al.*, 2008). This process is fundamental for sperm motility, which is acquired through oxidation of protein thiol (-SH) to form disulfides (S-S) and increases (-SH) and (-SH+S-S) ratio. It is interesting to note that, in this context, carnitine acts as a secondary antioxidant that repairs damage, thus improving glutathione and overall thiol status by exerting a sparing activity on thiol and methionine (Stradaioli *et al.*, 2004). This means that the highly significant correlations between carnitine and spermatozoa concentration could only be due to increase intracellular pool of carnitine.

Enzymatic antioxidants

GLUTATHIONE PEROXIDASE

GSH-Px is an important enzyme which plays a key role in detoxifying of any lipid peroxide which appears in the chicken sperm (Froman and Thurston 1981; Surai *et al.*, 1998). GSH-Px occupies particularly an important place in antioxidants due to its role in conversion of hydrogen peroxide into less harmful components (Olafsdottir and Reed 1988; Lin *et al.*, 2005; Eid *et al.*, 2006). This enzyme is considered qualitatively superior to catalase as it can maintain low cellular H_2O_2 (Lin *et al.*, 2005). GSH-Px and vitamin E are believed to be the primary components of antioxidant system, moreover, both these components act in synergy to protecting chicken sperm against oxidative stress (Eid *et al.*, 2006). GSH-Px is synthesised and regulated inside the body, however, dietary inclusion is also essential. GSH-Px works in cooperative manner with other enzymes like catalase and change in status of one component may affect that of the other (Lin *et al.*, 2005).

This system provides protection against lipid peroxides of cell membrane of sperm by arresting the progressive chain of lipid peroxidation. It also neutralises hydrogen peroxide; an initiator of lipid peroxidation. GSH-Px has been found both in poultry semen plasma and sperm (Surai *et al.*, 1998), however, there are species-specific differences in both activity and distribution of GSH-Px in avian semen. Surai *et al.* (1998) demonstrated that GSH-Px activity in the seminal plasma of turkey is the highest and the lowest in duck and goose.

GSH-Px is a selenium dependant enzyme (see above) and if this trace mineral is deficient in the diet, the semen may be vulnerable to the attack of lipid peroxidation. Surai *et al.* (1998) demonstrated that inclusion of selenium in the male diet significantly increases the concentration of GSH-Px activity in testes, spermatozoa and semen plasma. As a result, a significant decline in the susceptibility to lipid peroxides was observed. This protective effect was more pronounced in stored compared to fresh semen. Therefore, it is pertinent to say that selenium dependent GSH-Px (Se-GSH-Px) represents more than 75% of the total enzymatic activity of chicken sperm and more than 60% in the testes of cockerels. Surai *et al.* (1998) concluded that addition of 0.3 mg/kg of selenium increases GSH-Px activity and enhances protection against lipid peroxidation. Surai *et al.* (1998) found that inclusion of selenium in Rhode Island

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Red cockerels' diet had significant stimulating effect on GSH-Px activity in seminal plasma, spermatozoa and testes.

SUPEROXIDE DISMUTASE

SOD is an important component of seminal plasma and plays an essential role in striking balance between ROS generation and degradation by scavenging superoxide anion (Lin *et al.*, 2005; Eid *et al.,* 2006). SOD changes superoxide anion into hydrogen peroxide which is ultimately converted into water by catalase enzymes and GSH-Px (Lin *et al.*, 2005). Decrease concentration of SOD has been linked with abnormal sperm motility (de Lamirande and Gagnon, 1995).

Avian semen contains two types of SOD, mitochondrial form (Mn-SOD) and cytoplasmic form (Cu, Zn-SOD). There are species-specific differences in activity and distribution of SOD in avian semen. For example, Mn-SOD is the main form of this enzyme in avian spermatozoa, ranging from 57% (chicken) to 69% (guinea-fowl) of total SOD (Surai *et al.*, 1998). Mn-SOD is mainly present in spermatozoa while Cu, Zn-SOD is housed in seminal plasma (Surai *et al.*, 1998). This enzyme is synthesised and regulated endogenously and works in a cooperative manner with other enzymes like GHS-Px (Lin *et al.*, 2005). Significant amount of SOD is secreted by epididymis.

As seen in mammalian semen, SOD as an antioxidant enzyme in avian semen has also been identified (Froman and Thurston, 1981; Surai *et al.*, 1998). SOD scavenges both extracellular and intracellular superoxide anion and inhibits lipid peroxide of the cell membrane (Lin *et al.*, 2005; Eid *et al.*, 2006). It also acts against hydrogen peroxide by conjugating with catalase or GSH-Px (Jeulin *et al.*, 1989; Lin *et al.*, 2005). SOD prevents premature hyperactivation and capacitation caused by superoxide radicals before ejaculation (de Lamirande and Gagnon, 1995). Based on *in vitro* studies, Froman and Thurston (1981) observed lower SOD in samples, which suggested that turkey semen is more susceptible to oxidative stress than chicken semen.

Conclusions

Improving male fertility has been the major focus of poultry researchers in the last few decades. Better understanding of ROS has led to the development of various antioxidants as one of the remedies of low fertility. Current evidence suggests various preparations of antioxidants are beneficial for both *in vitro* and *in vivo* studies. However, definite conclusions cannot be drawn from the available studies, as the presence of ROS is not the only cause of infertility in male birds. The dosage and duration of dietary treatments are critical, particularly as investigators have not ascertained the required level of ROS for normal physiological purpose. Some of the studies have also shown ineffectiveness of using antioxidants on the sperm parameters. On the other hand, doses of high levels have either shown no effect on semen quality or have detrimental effects. Irrespective of this, there is ample evidence for the benefits of using antioxidants to improve semen quality which may have been impaired by exposure to elevated levels of ROS or low antioxidant defence status. Consensus is required on type, dosage and duration of antioxidants that should be used on studied parameters to augment semen quality. Furthermore, multicentric and larger numbers of samples in studies are required to gain more comprehensive insight into this essential problem.

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