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Sel-Plex® Improves Spermatozoa Morphology in Broiler Breeder Males

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Abstract: Sodium Selenite (SEL) has been the traditional source of Selenium (Se) in poultry diets, but Sel-Plex® (SP, a source of organic selenium in yeast protein, Alltech Inc.) has become widely used in several countries signaling its importance as a replacement for SEL. SP is equivalent or even superior to SEL in terms of gut absorption, performance, induction of whole body feathering and tissue retention. Therefore, it was important to extend our understanding of the influence of selenium on performance characteristics of poultry by comparing the influence of SEL or SP in broiler breeder roosters. In the first part of this investigation, 14-week-old roosters were fed diets that contained SEL, SP, or no supplemental selenium (deficient). Selenium-supplemented roosters produced semen at 19 weeks of age while selenium-deficient roosters did not produce semen until 26 weeks of age. Semen quality, as indicated by spermatozoal morphology, was best for SP-fed roosters and SEL-fed roosters produced semen with a quality that was intermediate between SP-fed and selenium-deficient rooster semen quality. In the second part of this investigation, adult roosters in a commercial setting were fed SEL at 0.3 ppm Se/kg of diet until they were 19 weeks of age and then half of the males on each of two farms were fed SP at 0.3 ppm Se/kg of diet. At 32 and 42 weeks of age, semen samples were evaluated via microscopy for quality based on spermatozoal morphology and spectrophotometric analysis to determine a sperm quality index, consisting of a composite determined by sperm motility and sperm density in the semen sample. The results from the laboratory trial and the field trial suggest that SP is superior to SEL as a source of selenium for broiler breeder males. This conclusion was further supported by histological evaluation of testicular tissues from roosters fed either no supplemental selenium, SP or SEL.

Key words: Sel-Plex®, selenium, broiler breeder, testes, semen, spermatozoa

INTRODUCTION

In many animal species, selenium can accumulate in high concentrations in endocrine glands. In the chicken, for example, it has been shown to accumulate (in decreasing order) in pituitary, pineal, adrenals, kidneys, pancreas, brain and ovary and testes (Vohra *et al*., 1973). Similarly, selenium can accumulate in the reproductive organs in mammals (Allan *et al*., 1999; Behne *et al*., 1988) with higher levels in the testes compared with other tissues (Behne *et al*., 1986; Hansen and Deguchi, 1996).

Since 1974, when selenium was permitted as a feed supplement, it has been clearly demonstrated that this trace element is essential for male fertility (Hansen and Deguchi, 1996). Behne *et al*. (1982) have shown that in conditions of selenium deficiency, rat testes will preferentially retain selenium. A deficiency in dietary selenium can result in decreased numbers of normal spermatozoa per ejaculate, decreased motility and decreased fertilizing capacity. These phenomena have been demonstrated in rodents, humans and poultry such as chickens, turkeys and ducks (Surai, 2000; Surai *et al*., 1998a,b; Surai *et al*., 2001). When semen samples were analyzed microscopically, it was evident that primary spermatozoal abnormalities were associated with spermatozoal head morphology and with the integrity of the midpiece, which contains the mitochondria that provide energy that allows for spermatozoal swimming and motility (Surai, 2000; Surai *et al*., 1998a,b; Surai *et al*., 2001). A spermatozoon that has an abnormal midpiece or head deformity is rendered permanently incapable of ovum fertilization (Froman *et al*., 1999; Sikka, 1996).

The objective of this investigation was to ascertain the contribution of dietary selenium on age of sexual maturation based on semen production and to assess the quality of that semen based on spermatozoal morphology.

MATERIALS AND METHODS

Animal welfare: This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee, which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and treatments: In part 1 of this investigation, young Cobb-500 males were maintained in a litter-covered floor pen on an 8L:16D photoperiod until 14 weeks of age when they were caged individually. The

photoperiod was raised to 12L:12D for two weeks after which the photoperiod was increased again to 16L:8D until the termination of the investigation when the roosters were 26 weeks old. The cockerels were fed a North Carolina Agricultural Research Service starter diet that provided 17% Crude Protein (CP) and 2925 Kcal/kg of Metabolizable Energy (ME) until the termination of the study. The 17% CP diet provided a background selenium level of approximately 0.28 ppm as determined chemically by the North Carolina Department of Agriculture and the starter diet was supplemented with sodium selenite (SEL) (0.2 ppm Se) only until the young roosters were placed into individual cages at 14 weeks of age. When the roosters were caged, they were divided into three groups of 10 birds each. Group A was fed a diet with no supplemental selenium and was considered to be the selenium-deficient diet. Group B was fed a diet to which Sel-Plex® (SP; a source of organic selenium as selenomethionine in yeast protein, Alltech, Inc., Nicholasville, KY 40356) was added at the level of 0.2 ppm Se. Group C was fed a diet to which SEL (inorganic selenium) was added at the level of 0.2 ppm Se. The supplemental levels of selenium were maintained in the three groups until the termination of the investigation. The total selenium levels in the diets are summarized in (Table 1).

At 17 weeks of age, all of the roosters were handled for the first time and the feathers around the cloaca were shortened to facilitate visualization of the vent area. Beginning at 18 weeks of age, each rooster was handled twice weekly and was stimulated via the abdominal massage method used to collect semen (Burrows and Quinn, 1935; 1937). A rooster was considered sexually mature when the handler was able to express semen after abdominal massage and stroking of the base of the tail, which will cause the protrusion of the genitalia and allow semen to be ejaculated onto the ejaculatory groove of the erect phallus after pressure is applied to the terminal storage depots of the vasa deferentia. No estimate of ejaculate volume was made, but semen was collected from all roosters at 26 weeks of age for evaluation of viability and quality by assessment of spermatozoal morphology using the eosin-nigrosin staining technique (Blom, 1950). Semen in a volume of 25 mL was collected directly from the ejaculatory groove and was placed in 1 mL of the eosin-nigrosin vital stain solution, gently mixed and allowed to rest for a period of 1 min. A drop of the semen-stain mixture was applied to a clean, oil-free microscope slide and using a blood smear movement using a clean slide over the semen-stain mixture on a second slide, the sample was dispersed across the length of the slide, which was then rapidly dried at 60°C on a covered slide warmer. The slides were evaluated using a 40X EF objective affixed to a Leitz OrthoPlan microscope. Dead spermatozoa were stained pink while viable spermatozoa were white. Additionally, various spermatozoal abnormalities were recorded for each semen sample evaluated. Spermatozoal abnormalities included bent midpiece with head of spermatozoa pointed backwards, ruptured midpiece, swollen midpiece, cork screw appearance involving the head and midpiece, coiled spermatozoa, detached head of spermatozoa usually broken at the interface between the neck and midpiece, multiple heads and malformed heads. A minimum of 500 cells per slide were counted in multiple fields randomly viewed across the whole slide.

At 26 weeks of age, when all roosters were considered to be sexually mature, after the final semen samples were collected from each bird, they were killed via asphyxiation in a carbon dioxide-filled chamber. The paired testes were dissected and weighed. The tunica albuginea was cut across in four locations on each testicle and the testes were fixed in 10% neutral buffered formalin for a period of three days before being processed for histological examination. Sections of the testes were embedded in paraffin, cut to a thickness of 5 μ, affixed to a microscope slide, dewaxed and stained with hematoxylin-eosin for evaluation of the seminiferous tubule development. Using a Nikon CoolPix 800 digital camera attached to a Leitz OrthoPlan microscope, photomicrographs of testicular sections were made to illustrate differences in microanatomy of the seminiferous tubules in testes from SP-, SEL- and no supplemental selenium-fed groups.

In the second part of this investigation, semen samples from Hubbard Ultra-Yield roosters in a commercial field setting were collected and evaluated to compare the effects of SP and SEL on semen quality as indicated histologically by spermatozoal morphology and spectrophotometric analysis (OptiBreed®, Alpharma Inc., Fort Lee, New Jersey 07024) through the composite measurement of sperm motility and sperm density that gave a Sperm Quality Index (SQI) for each sample (Parker *et al*., 2002). The male breeders had been managed as prescribed by the company's policies and had been fed 17% CP starter and grower diets containing SEL (0.3 ppm Se) until they were 21 weeks of age. At 21 wk of age, roosters in two of the four breeder houses on two separate farms involved in the investigation were fed SP at 0.3 ppm Se in the male diet, and the roosters in the other two houses continued to be fed SEL at 0.3 ppm Se in their diets. There were two sampling periods when the roosters were 32 and 42 weeks of age and at each sampling, groups of roosters fed either SP or SEL were penned separate from females in the breeder houses for a period of three days before semen samples were collected. A total of 20 semen samples were collected from roosters in each of the four houses at each sampling time. A quantity of the semen was taken first for the SQI measurements

Table 1: Influence of selenium source and level on chicken semen production and relative testes weight (±SEM)

Dietry selenium source	Basal Se (ppm)	Se Supplement (ppm)	Total Se (ppm)	Sexual Maturation (Wks)	Body weight 26 wks (kg)	Relative testes 26 wks (g/kg)
Basal	0.28	0.0	0.28	26	3.41±0.1[a]	4.58±1.35[b]
Sel-Plex	0.28	0.2	0.48	19	3.47±0.1[a]	9.85±0.10[a]
Selenite	0.28	0.2	0.48	19	3.41±0.1[a]	8.99±0.15[a]

[a,b,c]In a column, means with unlike superscripts differ significantly ($p < 0.05$), n = 10 for each selenium treatment.

(Parker *et al*., 2002) and the semen was then processed for histomorphometric assessment using the eosin-nigrosin staining procedure (Blom, 1950).

Statistics: All data from this completely randomized experimental design were analyzed using the general linear models procedure of SAS (SAS Institute, 1996). Percentages of the different spermatozoal forms assessed were normalized by converting them to angles (angle = arc sin √ %) for purpose of statistical analysis (Ostle, 1963). Statements of significance were based on $p \leq 0.05$ as indicated by analysis of variance.

RESULTS

Shown in Table 1 are the results for sexual maturation rate of roosters fed either no supplemental selenium, SP, or SEL. These results clearly indicate that selenium in general is necessary for male sexual maturation. Both SP- and SEL-supplemented groups were producing semen by 19 weeks of age, but roosters fed no supplemental selenium in their diet did not mature until 26 weeks of age. Even though sexual maturation did not occur in selenium-deficient roosters, their development was normal as indicated by body weight at 26 weeks of age. Body weight did not differ between the three experimental selenium source groups (Table 1). Relative testes weights did not differ between SEL- and SP-fed roosters, but relative testes weights of the selenium-deficient group at 26 weeks of age were significantly less than those of selenium-supplemented birds of the same age (Table 1).

At 26 weeks of age, when all roosters in the three experimental groups were sexually mature, final semen samples were collected from each bird, processed in the eosin-nigrosin vital stain, fixed on microscope slides and evaluated microscopically for spermatozoal morphological differences. Results for spermatzoal morphological characteristics shown in Table 2 clearly indicate that roosters fed no supplemental selenium had unusually large percentages of abnormal spermatozoa in their ejaculates and those percentages were significantly greater than comparable percentages found in either SEL- or SP-fed roosters. The supplementation of SEL improved normal spermatozoal percentages over the no supplemental selenium roosters by more than 30% and SP caused the increase to be more than 40% over the no supplemental selenium group. The majority of the abnormalities in the three selenium source groups were concentrated in the area of the midpiece. In the no supplemental selenium group, midpiece abnormalities accounted for 21.2% of the total number of spermatozoa counted, but SEL supplementation reduced midpiece abnormalities to 6.7% while SP further reduced midpiece abnormalities to 0.8%. A similar distribution of the corkscrew head/midpiece abnormality was found in the group fed no supplemental selenium (15.4%), SEL (1.8%) and SP (0.2%). For head abnormalities, the group fed no supplemental selenium had 5.5% of total, SEL had 2.1% of total and SP had only 0.3% of total. The head abnormalities had several categories and detached head, in which the head of the spermatozoal cell was broken at the neck and midpiece interface, was the predominant category found in this investigation. In terms of detached heads, the groups fed no supplemental selenium (1%) and SEL (1.1%) were similar; in contrast SP suppressed this abnormality to only 0.1% (Table 2).

Histological sections from the testes revealed that there were few differences between SP- and SEL-fed roosters at 26 weeks of age. The SP-fed roosters had developed a well-defined hierarchy of spermatogenic cells exhibiting spermatogonia, spermatocytes, spermatids and spermatozoa (Fig. 1).

The testes from SEL-fed roosters also had a hierarchy of spermatogenic cells similar to the cellular development in testes from SP-fed roosters, but in testes from SEL-fed roosters, subjectively, it appeared that there were more spermatids in each hierarchy than found in testes from SP-fed roosters. When the testicular sections from the SP-fed roosters were compared with the testes from roosters fed no supplemental selenium, very obvious morphological differences could be readily observed (Fig. 2).

First, spermatid numbers in roosters fed no supplemental selenium were apparently in greater quantities than in either the SP- or SEL-fed roosters. A similar pattern was associated with greater numbers of spermatogonia and spermatocytes in the group fed no supplemental selenium compared with those fed selenium. The hierarchical arrangement of spermatogenic cells in the seminiferous tubules of the roosters fed no supplemental selenium was not as apparent as that found in selenium-fed roosters. Subjectively, there appeared to be more but smaller Sertoli cells associated with the seminiferous tubules of the roosters fed no supplemental selenium compared with those fed selenium in general. Another major

Table 2: Spermatozoal abnormalities found in semen from roosters given fed either sodium selenite, Sel-Plex® or no supplemental selenium

Spermatozoal Form	Basal (%)	Selenite (%)	Sel-Plex (%)
Normal	57.9±6.2[c]	89.4±3.0[b]	98.7±2.7[a]
Midpiece Abnormalities			
Bent	18.7±2.3[a]	6.2±0.5[b]	0.7±0.1[c]
Swollen	1.6±0.2[a]	0.4±0.1[b]	0.1±0.1[c]
Ruptured	0.9±0.1[a]	0.1±0.1[b]	0.0±0.0[b]
Cork Screw	15.4±2.6[a]	1.8±0.3[b]	0.2±0.1[c]
Head Abnormalities			
Swollen	1.3±0.2[a]	0.2±0.1[b]	0.2±0.1[b]
Coiled	3.2±0.4[a]	0.8±0.1[b]	0.0±0.0[c]
Detached Head/Other	1.0±0.1[a]	1.1±0.1[a]	0.1±0.1[b]

[a,b,c]In a row, means with unlike superscripts differ significantly ($p \leq 0.05$), n = 10 for each selenium treatment

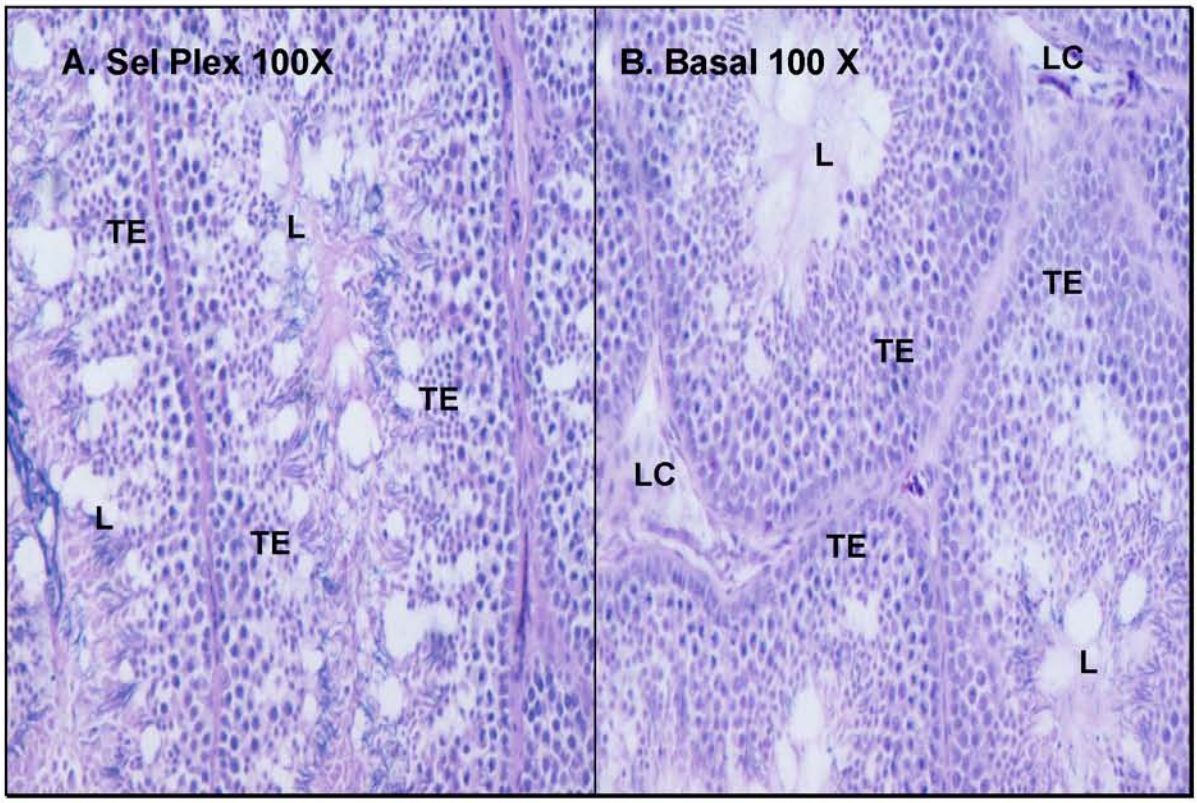

Fig. 1: Cross section (100 x magnification) of seminiferous tubules, showing the Lumen (L) and Tubule Epithelium (TE) and Leydig Cells (LC) from roosters fed Sel-Plex® (A) or no supplemental selenium (B)

morphological difference that could be readily ascertained was that the seminiferous tubules of the no selenium-fed roosters appeared to contain more spermatogenic cells as undifferentiated and differentiated spermatogonia and primary spermatocyte forms compared with those fed selenium (Fig. 1 and 2).

In the second part of this investigation, semen samples from commercial broiler breeder roosters in multiplier flocks were examined histologically for distribution of normal, dead and abnormal spermatozoal categories (Table 3). SP-fed roosters had significantly greater percentages of normal spermatozoa at both 32 and 42 weeks of age compared with roosters that had been fed SEL for their whole life (Table 3).

Additionally, at both 32 and 42 weeks of age, SP-fed roosters had significantly lower percentages of dead spermatozoa, spermatozoa with midpiece abnormalities, corkscrew head abnormalities and other abnormalities (mostly detached heads at the neck-midpiece interface) compared with semen samples evaluated from roosters that had been fed SEL (Table 3). Furthermore, the SQI (provided by Dr. C.D. McDaniel, Mississippi State University, Department of Poultry Science, Starkville, MS) was significantly greater for

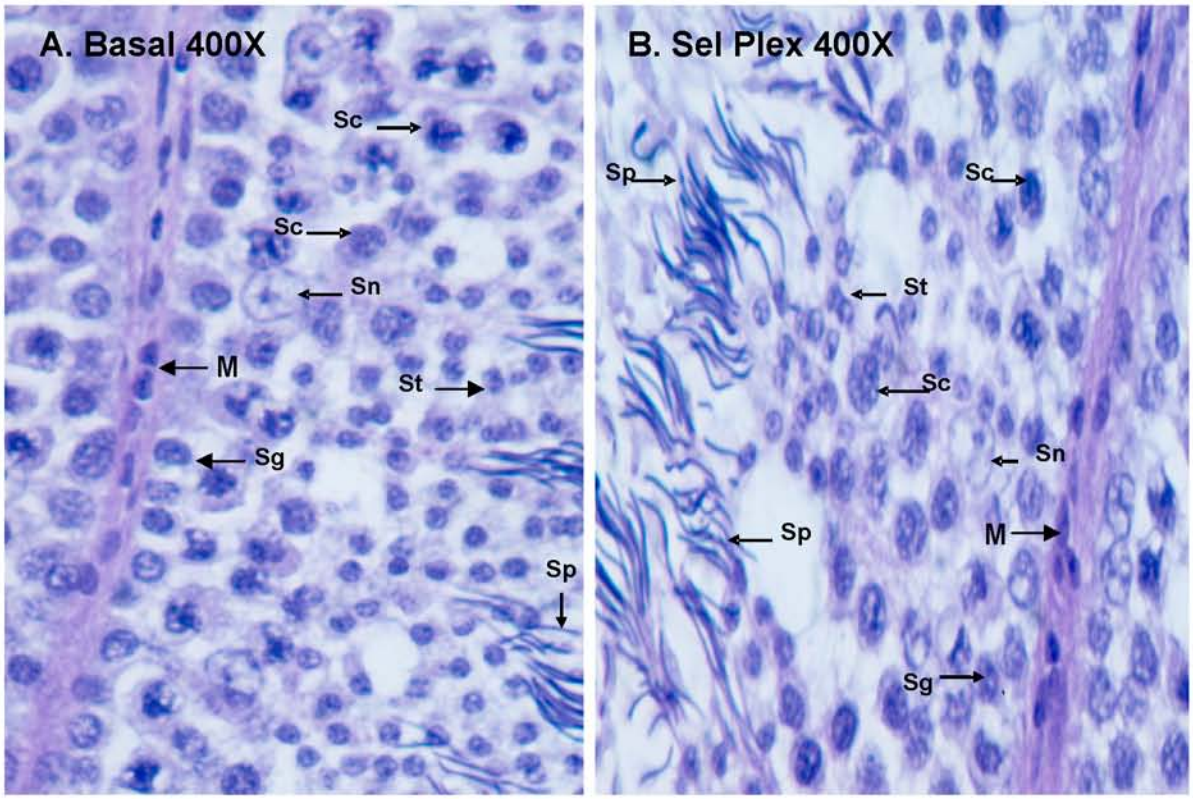

Fig. 2: Cross section (400 x magnification) of testes from roosters fed no suppelemental selenium (A) or Sel-Plex® (B). Sg-spermatogonia; Sc-spermatocytes; Sn-Sertoli cells; Sp-spermatozoa; St-spermatids; M-myoid cells

Table 3: Influence of 0.3 ppm selenium source (Sel-Plex® vs. sodium selenite) on distribution of normal and abnormal spermatozoal categories in commercial Hubbard Ultra-Yield broiler breeder roosters at 32 and 42 weeks of age and the relationship of spermatozoal functional anatomical characteristics with the sperm quality index

Selenium Source	Spermatozoal Category (%)	32 Weeks of Age	42 Weeks of Age	SQI
Sel-Plex	Normal	98.01±1.13^{a,x}	97.65±1.15^{a,x}	297^{a}
	Dead	0.42±0.10^{b,x}	0.56±0.23^{b,x}	
	Abnormal Midpiece	0.51±0.25^{b,x}	0.76±0.21^{b,x}	
	Corkscrew Head	0.40±0.02^{b,x}	0.58±0.24^{b,x}	
	Other	0.24±0.01^{b,x}	0.41±0.06^{b,x}	
Sodium Selenite	Normal	91.93±1.24^{b,x}	85.39±3.34^{b,y}	254^{b}
	Dead	1.31±0.22^{a,y}	1.94±0.21^{a,x}	
	Abnormal Midpiece	3.78±0.25^{a,y}	6.21±0.82^{a,x}	
	Corkscrew Head	1.33±0.31^{a,y}	3.19±0.71^{a,x}	
	Other	1.96±0.36^{a,y}	3.22±0.42^{a,x}	

^{a,b}Within Weeks of Age, comparison of the influence of Sel-Plex to sodium selenite on specific spermatozoal categories, means with unlike superscripts differ significantly ($p \leq 0.05$).

^{x,y}Comparison of the influence of age on specific spermatozoal categories, means with unlike superscripts differ significantly ($p \leq 0.05$).

N = 40 for each selenium source

those roosters fed SP compared with those fed SEL (Table 3). There was a significant age X treatment interaction for all categories of spermatozoa evaluated. This age X treatment interaction indicated that SEL-fed roosters were decreasing their output of normal spermatozoa as they aged from 32-42 weeks of age, and as the SEL-fed birds aged the percentages for dead spermatozoa and those with abnormal midpieces, corkscrew heads and detached heads, also increased significantly, however these changes were not observed in roosters fed SP from 21-42 weeks of age (Table 3).

DISCUSSION

Spermatozoa contain some of the highest concentrations of selenium found on a cellular basis in the body. Much of the selenium in the testes can be found concentrated into specific selenoproteins and among those selenoproteins, Sperm Mitochondria-

Table 4: Spermatozoal abnormalities found in semen from lines of chickens selected for divergent body weight[1]

Sperm Forms	High Weight Line	Low Weight Line
Normal	54.04±4.49[b]	67.95±3.57[a]
Abnormal Midpiece	18.44±0.94[a]	13.33±0.98[b]
Bent Midpiece	9.19±0.95[a]	7.25±0.74[a]
Cork Screw	6.58±0.71[a]	4.90±0.67[a]
Fragment	3.97±0.75[a]	2.70±0.69[a]
Other Abnormal	4.78±0.84[a]	3.87±0.79[a]

[a,b]In a row, means with unlike superscripts differ significantly ($p \leq 0.05$).
[1]Diets were not supplemented with Selenium; age of sexual maturity was 26-28 weeks for males with HWL males maturing faster than LWL males. Adapted from Edens (1970)

associated Cysteine-rich Protein (SMCP), previously described as Mitochondrial Capsule Selenoprotein (MCS) and Mitochondrial Capsule Protein (MCP), is a major structural element of the mitochondria in the midpiece of the spermatozoon tail (Kleene, 1994; Ursini *et al*., 1999). The SMCP has a high concentration of selenium contained in phospholipid hydroperoxide glutathione peroxidase (PH-GSHpx; Ursini *et al*., 1999), which makes up approximately 50% of the SMCP that acts as a structural protein and embeds the mitochondrial helix in the mature spermatozoon. However, in the testis, the PH-GSHpx acts as a powerful antioxidant in the developing spermatids and spermatozoa (Ursini, *et al*., 1999). The dual role played by PH-GSHpx in maturation and in the mature spermatozoon partially explains the mechanical instability of the mitochondrial midpiece of spermatozoa from selenium-deficient animals similar to the results observed in this investigation.

In part one of this investigation, the roosters were fed a basal diet that contained approximately 0.28 ppm of selenium from different feed ingredients. The background levels of dietary selenium ostensibly would have been sufficient to maintain a healthy selenium-dependent redox status if all of that selenium had been available to the roosters. Based on the results of the first part of this study, all the roosters should have been equivalent in their abilities to grow and mature, but development of the testes and their ability to produce spermatozoa were compromised in those animals fed the basal diet with no supplemental selenium. Nevertheless, supplementation of the basal diet with SP and SEL corrected the problem in terms of facilitating testicular development and spermatozoa production. The use of SEL, although inducing a highly significant response for testicular development, also allowed for smaller numbers of normal spermatozoa and significantly greater numbers of abnormal forms, which was evident also in the results from field research with a commercial multiplier flock fed SEL compared with SP-fed roosters.

Spermatozoal abnormalities, especially in the midpiece of the tail of sperm, found in association with selenium deficiency, have been recognized for many years (Wu *et al*., 1979). The midpiece abnormality is associated with a deficiency in the selenium-rich SMCP that provides structural integrity to the midpiece (Kleene, 1994; Ursini *et al*., 1999). Therefore, one must extend the observations made in mammalian models and conclude that the midpiece of avian spermatozoa also contains SMCP or SMCP-like protein that is compromised by selenium deficiency leading to increased numbers of bent spermatozoa and other abnormalities related to the midpiece. It was surprising that there were more spermatozoal midpiece abnormalities associated with SEL-supplemented roosters compared with the SP-supplemented roosters (Table 2 and 3), but given the potential of oxidative stress associated with the feeding of SEL, it is not infeasible for these abnormalities to occur with the use of SEL. SEL has a documented pro-oxidant influence in all animals tested including humans (Hafeman *et al*., 1974; Csallany and Menken, 1986; Spallholz, 1997; Terada *et al*., 1999). As a pro-oxidant, SEL has the potential to facilitate the production of Reactive Oxygen Metabolites (ROM) and nitrogen metabolites causing lipid peroxidation and ultimately promoting the breakdown of cell membranes that leads to spermatozoal abnormalities. Surai (2000) has discussed the antioxidant system in avian testes and semen and established that the concentration of selenium found in seminal plasma and spermatozoa is finely regulated and if those concentrations are decreased, the function of the spermatozoon will be negatively affected. Most of the selenium in spermatozoa is contained in SMCP as PH-GSHpx but a substantial portion of the selenium is in GSHpx and other selenium-containing proteins in the mitochondria and in seminal plasma. It appears that because of the high rate of metabolism demonstrated in spermatozoa, a significant production of ROM can be generated and these along with lipid peroxides must be reduced to prevent damage to spermatozoa (Surai, 2000; Surai *et al*., 2001; Surai *et al*., 1998a,b). This need partially explains the necessity for the finely controlled high levels of selenium found in spermatozoa and seminal plasma. Yet the spermatozoa are subject to the damaging effects of high concentrations of polyunsaturated fatty acids that can be readily oxidized to peroxides in the aerobic environment of the testis, semen and in the uterovaginal sperm host glands in hens (Surai, 2000; Surai *et al*., 2001; Surai *et al*., 1998a,b) and increased levels of SEL in the diet can promote this oxidation. A balance between pro-oxidants and antioxidants must be established in tissues and body fluids with high metabolic rate to ensure the survival and functioning of cells in those aerobic environments. Oxidative stress, caused by peroxidation of polyunsaturated fatty acids in these tissues and fluids, can lead to irreparable damage in the cell membranes of the spermatozoon and render it incapable of ovum

Table 5: Duration of fertility of lines of chickens selected for divergent body weight[1]

Fertility	HWL x HWL	HWL x LWL	LWL x HWL	LWL x LWL
7 d, % Fertile	48.28±3.79[c]	56.82±3.78[b]	58.29±3.65[b]	64.94±3.80[a]
18 d, % Fertile	33.68±2.91[c]	38.84±3.00[b]	42.32±2.93[ab]	48.42±2.82[a]

[a,b,c]In a row, means with unlike superscripts differ significantly ($p \leq 0.05$).

[1]Diets were not supplemented with selenium; age of sexual maturity was 24-26 weeks for females and 26-28 weeks for males. HWL females matured earlier than LWL females. Adapted from Edens (1970)

fertilization, the only function of the sperm cell. Sies (1993) indicated that the physiological and pharmacological strategies for antioxidant defense are organized into the categories of prevention, interception, and repair. In the context of antioxidant defense, selenium and the glutathione system act primarily at the level of interception and this leads to transfer of the pro-oxidant away from sensitive compartments in cells. Thus, if the pro-oxidant/antioxidant balance shifts toward the pro-oxidant condition on the testis, semen, or in the hen's uterovaginal or infundibular sperm host glands, the function of spermatozoa will be rapidly degraded leading to male infertility.

Experimental evidence indicates that spermatozoal morphology is severely altered in poultry that have been fed selenium-deficient diets. In dietary selenium deficiency, the primary spermatozoal abnormality was shown to be a bent midpiece in which the spermatozoa swim with the acrosome pointed backwards essentially reducing the fertilizing capacity of the sperm cell to 0%. In poultry, there has been little evidence presented to show that selenium deficiency has affected fertilizing capacity of spermatozoa. However, between 1968 and 1970, studies conducted at Virginia Polytechnic Institute and State University (Edens, 1970) provided evidence that dietary selenium deficiency, which was standard in breeder diets before 1974, did indeed cause a problem in White Plymouth Rock breeders that had been selected divergently for high or low body weight (Table 4). By today's standards, both lines were experiencing reproductive problems, but the problems in the High Weight Line (HWL) were more severe than in the Low Weight Line (LWL) (Table 5). The data indicated that fertility was poorest in the HWL X HWL cross and the best fertility was in the LWL X LWL cross. Crossing HWL males with LWL females improved male fertility, but crossing LWL males with HWL females depressed male fertility. Thus, at that time, it was ascertained that genetic factors in both males and females could adversely affect fertility.

However, when that thesis work was conducted, scientists in general did not know about the beneficial influence of selenium on reproductive fitness in both genders of all poultry species. When selenium was added to poultry diets in 1974, reproductive fitness improved almost immediately and has remained at those elevated levels. With the use of organic selenium in Sel-Plex, it can be demonstrated that genetic breeding potential can be more closely addressed in commercial flocks.

It is important to keep in mind that there was no supplemental selenium fed to those HWL and LWL males in 1968. The selenium that they had available to them had to come from feed grains, meat and bone meal and from fish meal. Those selenium sources were most certainly providing some forms of organic selenium, but all organic selenium is not available, especially from meat and bone meal and from fish meal similar to the background level of selenium fed to roosters in part one of this investigation. If feed grains were grown in regions with low selenium levels in soils, those grains, the primary source of selenomethionine, selenocysteine and selenocystine, would also be low in selenium content rendering the birds to a state of selenium deficiency. In part one of this investigation, young broiler breeder roosters were induced to come into sexual maturity (19 weeks of age) long before the optimal time for sexual maturation recommended for commercial breeders. Both SEL- and SP-fed roosters began to produce semen at 19 weeks of age, which was very early, but it was not until 26 weeks of age that the basal-fed males produced semen. Additionally, it was evident that basal-fed males had delayed sexual maturation based upon development of secondary sex characteristics (comb and wattles), testicular size at 26 weeks of age and delayed onset of crowing even under the influence of photo-stimulation. The signs of secondary sexual development in the selenium-supplemented roosters was an indication that hormonal development was advanced at an early age (19 weeks of age) but not in the selenium-deficient roosters. Behne *et al.* (1987) observed that testicular selenium content is regulated by Follicle-stimulating Hormone (FSH), which is also responsible for spermatogenesis in the seminiferous tubules. Burk (1978) found aspermatogenesis in first-generation rats fed a selenium-deficient diet and Sprinker *et al.* (1971) reported consistent testicular atrophy and aspermatogenesis in second generation selenium-deficient rats.

Furthermore, Leutinizing Hormone (LH) regulates the Leydig cells that produce testosterone in the testes and when Luteinizing Hormone-releasing Hormone (LHRH) was fed to selenium-deficient rats, serum testosterone levels increased at a slower rate in selenium-deficient rats compared with controls. Behne *et al.* (1987) concluded that selenium deficiency affected Leydig cells and suggested a biological function of selenium in the steroidogenic cells of the testes. Therefore, the

advanced development of secondary sex characteristics in selenium-supplemented roosters in the first part of this investigation was normal, whereas delayed development in roosters fed the basal diet with no supplemental selenium was a sign of selenium deficiency that impaired testosterone secretion. However, these observations are contrasted with the conclusions of Combs and Combs (1986) who stated that there is no evidence that selenium deficiency influences male reproduction in poultry. Data from the investigation reported herein clearly indicate that there is a selenium influence on male reproductive potential in roosters. The histology of the testes from the roosters involved in part one of this investigation was also studied. The histological results showed that those males fed no supplemental selenium were not as committed to spermatozoal formation in the same time frame as were those fed SP (Fig. 1 and 2). In testes from selenium-deficient roosters, there were more Sertoli cells and fewer hierarchies of spermatogonia that were committed to spermatid formation compared with roosters fed SP. Leydig cell formations also appeared to be less developed in the roosters fed no supplemental selenium. These observations conform with those made in selenium-deficient mammalian models (Burk, 1978; Behne *et al*., 1987) and partially explain the delayed sexual maturity in the roosters fed no supplemental selenium.

The observation that there were more but smaller Sertoli cells in the testicular sections from roosters fed no supplemental selenium compared with SP is very important. González-Morán *et al*. (2008) have shown that Sertoli cell numbers decrease with maturation of roosters, reaching the lowest numbers at the time of maximized testicular weight and then increase again as the rooster ages and fertility declines.

Conversely, the number of germ cells was inversely related to the Sertoli cell numbers. According to Rosenstrauch *et al*. (1994), Sertoli cells function to hold mature spermatozoa in place in the seminiferous tubules and regulate the release of the sperm cells. In low fertility roosters, the Sertoli cells are smaller than in high fertility roosters and their numbers are elevated in low fertility roosters. The mature spermatozoa are retained in the seminiferous tubules of young roosters, released readily in mature roosters to maximize fertility and are retained in aged roosters leading to reduced fertility. This scenario is similar to that reported herein with the difference being attributed simply to the lack of available dietary selenium. In low fertility roosters, Rosenstrauch *et al*. (1994) suggest that Sertoli cells lose their ability to regulate the release of mature sperm cells. The retention of spermatozoa in selenium-deficient roosters in this study (Fig. 1 and 2) suggests that the metabolism of Sertoli cells might be affected by selenium and that reduced metabolism of the Sertoli cells might be associated with increased numbers of abnormal spermatozoa that are released. This concept cannot be substantiated with the data in this report and additional work is required. Nevertheless, it is apparent that selenium deficiency can and does lead to increased numbers of abnormal spermatozoa being released even in mature roosters. Perhaps, SEL supplementation plays a negative role, because SEL, a known pro-oxidant, has the ability to cause membrane damage due to lipid peroxidation. Part of the increased rate of release of abnormal spermatozoa might be attributed to lack of PH-GSHpx in selenium-deficient roosters. Weakened and decreased concentration of selenium-dependent structural proteins in the midpiece of the spermatozoa results in most of the observed abnormalities. Even historical evidence suggests that selenium deficiency has been the cause of decreased semen quality in roosters (Edens, 1970).

REFERENCES

Allan, C.B., G.M. Lacourciere and T.C. Stadtman, 1999. Responsiveness of selenoproteins to dietary selenium. Ann. Rev. Nutr., 19: 1-16.

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

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

Behne, D., H. Hilmert, S. Scheid, H. Gessner and W. Elger, 1988. Evidence for specific selenocysteine target tissues and new biologically important selenoproteins. Biochem. Biophys. Acta., 966: 12-21.

Behne, D., T. Höfer-Bosse and W. Elger, 1987. Selenium and hormones in the male reproductive system. Pages 733-739. In: Selenium in Biology and Medicine. Part B.G.F. Combs, Jr., J. E. Spallholz, O.A. Levander and J.E. Oldfield Eds. Van Nostrand Reinhold Company, New York, New York.

Blom, E., 1950. A one-minute live-dead sperm stain by means of eosin-nigrosin. Fert. Ster., 1: 176-177.

Burk, R.J., 1978. Selenium in nutrition. World Rev. Nutr. Diet., 30: 88-106.

Burrows, W.H. and J.P. Quinn, 1935. A method of obtaining spermatozoa from the domestic fowl. Poult. Sci., 14: 251-254.

Burrows, W.H. and J.P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and turkey. Poult. Sci., 16: 19-24.

Combs, Jr., G.F. and S.B. Combs, 1986. The Role of Selenium in Nutrition. Academic Press, Boca Raton, Florida.

Csallany, A.S. and B.Z. Menken, 1986. Effect of dietary selenite on hepatic organic solvent-soluble lipofuscin pigments. J. Am. Coll. Toxicol., 5: 79-85.

Edens, F.W., 1970. Reproductive Characteristics in Lines of Chickens Selected for High and Low Juvenile Body Weight. Master of Science Thesis, The Graduate School, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Froman, D.P., A.J. Feltman, M.L. Rhoads and J.D. Kirby, 1999. Sperm mobility: A primary determinant of fertility in the domestic fowl *(Gallus domesticus*). Biol. Reprod., 61: 400-405.

González-Morán, M.G., C. Guerra-Araiza, M.G. Campos, and I. Camacho-Arroyo, 2008. Histological and sex steroid hormone receptor changes in testes of immature, mature and aged chickens. Domestic Anim. Endocrinol., 35: 371-379.

Hafeman, D.G., R.A. Sunde and W.G. Hoekstra, 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.

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

Kleene, K.C., 1994. The mitochondrial capsule selenoprotein- a structural protein in the mitochondrial capsule of mammalian sperm. Pages 135-148. In: Selenium in Biology and Human Medicine, R.F. Burk, Eds. Springer-Verlag, New York, New, York.

Ostle, B., 1963. Statistics in Research. Iowa State University Press. Ames, Iowa.

Parker, H.M., A.G. Karaca, J.B. Yeatman, L.R. Frank and C.D. McDaniel, 2002. Fertility of broiler breeders following categorization by the OptiBreed® Sperm Quality Index when hens are inseminated with a constant number of sperm. Poult. Sci., 81: 239-245.

Rosenstrauch, A., A.A. Degen and M. Friedländer, 1994. Spermatozoa retention by Sertoli cells during decline in fertility in aging roosters. Biol. Reprod., 50: 129-136.

SAS® Institute, 1996. A User's Guide to the Statistical Analysis System. SAS® Institute, Cary, NC.

Sies, H., 1993. Strategies of antioxidant defense. Eur. J. Biochem., 215: 213-225.

Sikka, S.C., 1996. Oxidative stress and role of antioxidants in normal and abnormal sperm functions. Front. Biosci., 1: e78-e86.

Spallholz, J.E., 1997. Free radical generation by selenium compounds and their pro-oxidant toxicity. Biomed. Environ. Sci., 10: 260-270.

Sprinker, L.H., J.R. Harr, P.M. Newberne, P.D. Whanger, and P.H. Weswig, 1971. Selenium deficiency lesions in rats fed vitamin E-supplemented rations. Nutr. Rep. Int., 4: 335-340.

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

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

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

Surai, P.F., N. Fujihara, B.K. Speake, J-P. Brillard, G.J. Wishart and N.H.C. Sparks, 2001. Polyunsaturated fatty acids, lipid peroxidation and antioxidant protection in avian semen. Asian-Aust. J. Anim. Sci., 14: 1024-1050.

Terada, A., M. Yoshida, Y. Seko, T. Kobayashi, K. Yoshida, M. Nakada, K. Nakada, H. Echizen, H. Ogata and T. Rikihisa, 1999. Active oxygen species generation and cellular damage by additives of parenteral preparations: Selenium and sulfhydryl compounds. Nutr., 15: 651-655.

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

Vohra, P.C.M. Johnson, L.Z. McFarland, T.D. Siopes, W. O. Wilson and C.M. Winget, 1973. Role of selenium in neural physiology of avian species. 2. The distribution of ^{75}Se from injected selenomethionine-^{75}Se in the testes and its fate in livers of chickens. Poult. Sci., 52: 644-647.

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