GROWTH PERFORMANCE, CARCASS TRAITS, AND REPRODUCTIVE CHARACTERISTICS IN BOARS FED DIETS SUPPLEMENTED WITH AN ORGANIC SOURCE OF SELENIUM

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ABSTRACT

The objectives of this study were to assess growth and reproductive performance of boars fed a diet supplemented with organic selenium (Se). Crossbred boars received one of three treatments: I. basal diet with no supplemental Se, II. basal diet supplemented with 0.3 ppm organic Se (Sel-Plex), and, III. basal diet supplemented with 0.3 ppm sodium selenite. Nursery (n = 13 pens/treatment) boar performance was not affected ($P > 0.1$) by diet and only grow-finish (n = 11 pens/treatment) G:F was greater ($P < 0.06$) for Sel-Plex (0.378) compared with selenite (0.368) or control (0.363) boars. At 15-mo of age semen was collected from boars (n = 10/treatment) over 5-d. Semen quality declined over time, but the negative impact day had on sperm motility was less pronounced with Sel-Plex boars. Effects of treatment x day were detected for progressively motile ($P = 0.02$) and rapidly moving ($P = 0.03$) spermatozoa, sperm path velocity (VAP; $P = 0.05$), and average velocity (VSL; $P = 0.05$). At 17-mo of age, semen was collected from boars (n = 10/treatment), extended and stored over 10-d. Although semen quality decreased over time, sperm from Sel-Plex boars resisted the negative effects of day on sperm motility and pH. Effects of treatment x day were detected for percent motile spermatozoa ($P < 0.01$), static spermatozoa ($P < 0.01$), VAP ($P = 0.06$), amplitude of head displacement (ALH; $P = 0.02$), straightness ($P = 0.01$), and pH ($P < 0.01$). At 23-mo of age, semen was collected (day 0) from boars (n = 6/treatment), extended, stored and evaluated at d 1 and 8 using in vitro fertilization. Dietary Se treatment failed to affect ($P < 0.05$) in vitro fertilizing rates of boars. In summary, dietary supplementation with Sel-Plex enhanced G:F in grow/finish boars. Dietary Sel-Plex supplementation may decrease the effects that stressors, such as intensive semen collection or semen storage, have on boar sperm characteristics such as sperm motility. The mechanisms for these responses remain to be elucidated.

Key words: boar, carcass, fertility, growth, selenium, semen

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LISTS OF ABBREVIATIONS

Abbreviation	Definition
ADFI	average daily feed intake
ADG	average daily gain
AFABP	adipocyte fatty acid binding protein
AI	artificial insemination
ALH	amplitude of linear head displacement
AP-1	activator protein-1
AR	androgen receptor
ATP	adenosine triphosphate
AV	artificial vagina
BCF	beat cross frequency
BTS	Beltsville thawing solution
BW	body weight
CASA	computer-assisted sperm analysis
Cm	Centimeter
D	day
D1	type 1 iododeiodinase
D2	type 2 iododeiodinase
D3	type 3 iododeiodinase
DM	dry matter
DNA	deoxyribonucleic acid
eEFSec	selenocysteine-specific elongation factor
EU	European Union
Exp.	Experiment
FDA	U.S. Food and Drug Administration
FSH	follicle stimulating hormone
G	Grams
G:F	Gain:feed ratio
GH	growth hormone
GnRH	gonadotropin-releasing hormone
GPx	glutathione peroxidase
GPx1	cytosolic or classical glutathione peroxidase 1
GPx2	plasma glutathione peroxidase 2
GPx3	gastrointestinal glutathione peroxidase
GPx4	phospholipid hydroperoxide glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidized glutathione
H	Hour
HCW	hot carcass weight
HSP	Heat shock protein
i.m.	Intramuscular
IGF-1	insulin-like growth factor
IU	international units
IVF	in vitro fertilization
kDa	Kilodalton

Kg	Kilogram
L -	levo-
LH	Luteinizing hormone
LHCGR	Luteinizing hormone-human chorionic gonadotropin receptor
LIN	Linearity
LM	Longissimus muscle
LPL	Lipoprotein lipase
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
Mn-SOD	Superoxide dismutase
MPN	male pronucleus
mRNA	Messenger ribonucleic acid
NFκB	nuclear factor kappa B
ng	nanogram
nmol	Nanomolar
NO	nitric oxide
NOS	nitric oxide synthase
ºC	degree Celsius
P	Probability
PCR	polymerase chain reaction
ppb	parts per billion
ppm	parts per million
PUFA	Polyunsaturated fatty acid
r	simple correlation coefficient
r^2	simple coefficient of determination
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
s	Second
s.c.	Subcutaneous
SBP2	SECIS binding protein 2
Se	Selenium
SECIS	selenocysteine insertion sequence
SeCys	Selenocysteine
SeMet	Selenomethionine
SeP	selenoprotein P
Ser	Serine
snGPx	sperm nucleus glutathione peroxidase
STAR	steroidogenic acute regulatory protein
T_3	Triiodothyronine
T_4	thyroxine, tetraiodothyronine
TBARS	thiobarbituric acid reactive substances
TEGT	testis enhanced gene transcript

TRH	thyroid releasing hormone
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone
VAP	average path velocity
vs.	Versus
VSL	straight-line velocity
wk	Week
x	multiplied by
γ-GCS	γ-glutamyl cysteine synthetase

GRANTING INFORMATION

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DEDICATION

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CHAPTER ONE

INTRODUCTION

The use of artificial insemination (AI) within the U.S. swine industry has increased so substantially over the past two decades that it is now estimated that 80% of pork producers utilize this reproductive technology.. To remain efficient, however, swine AI programs will need to utilize tools to enhance semen production and fertility in boars. Nutritional strategies, such as supplementing boar diets with selenium (Se), may be one method that producers can use to enhance boar fertility. Enhancing the reproductive rate by a mere 3% could result in more than 3,000,000 or more pigs born each year (Senger, 2003).

Selenium is an essential trace mineral that has been implicated in an assortment of biochemical and physiologic processes. Both deficiency and excess of Se has been associated with a myriad of problems, including growth and reproductive problems, and supplementation with Se has been reported to improve reproductive performance in numerous species (Kohrle et al., 2005). Although negative effects of Se deficiency are seen in both the male and female reproductive systems, Maiorino et al., 1999 states that the male reproductive system is more sensitive to Se deficiency. A review by Surai (2006) established that while clinical Se deficiency is rarely seen in the swine industry, subclinical Se deficiency could be responsible for reduced growth and reproductive performance. Thus, the true impact of subclinical Se deficiency is unknown.

Swine diets are typically supplemented with 0.3 ppm Se as sodium selenite, the maximum rate allowed by the U.S. Food and Drug Administration (FDA, 2003). The primary reason the FDA limited supplementation rates to 0.3 ppm Se were because of environmental concerns, particularly in Se accumulation in watersheds and its effect on reproduction in

waterfowl. Current regulations allow producers to use either inorganic or organic forms of Se supplementation in livestock diets. Mahan (2001) stated that most producers use an inorganic Se source, in the form of sodium selenite, because of its cost effectiveness.

Research has discovered, however, that the inorganic and organic sources of Se vary greatly in biological availability and effectiveness. Mahan and Kim (1996) suggested that Se from inorganic sources, such as sodium selenite, are not as biologically available or effective compared to the organic selenomethionine that is indigenous in cereal grains Mahan and Parrett (1996) discovered that pigs fed organic Se (Sel-Plex) have increased tissue retention and reduced excretion of Se compared to pigs fed an inorganic Se (sodium selenite). Furthermore, pigs supplemented with inorganic Se (sodium selenite) excrete more Se through the urine, whereas pigs supplemented with organic Se (Sel-Plex) excrete more Se through their feces (Mahan and Parrett, 1996). Although the form of Se supplementation does not make a difference in current FDA regulations the form of Se supplementation should make a difference in the environmental impact when manure is applied as fertilizer onto crop fields.

Compared to males of other species, relatively little is known about the effect of Se supplementation on the boar. Marin-Guzman et al. (1997, 2000a,b) reported increased Sertoli cell numbers, testicular sperm reserves, sperm motility and morphology in addition to increasing fertilization rates when gilts were inseminated with semen from boars receiving diets supplemented with 0.5 ppm sodium selenite. A Polish study later demonstrated that ejaculates from boars fed an organic source of Se (0.2 ppm Se) and 60 IU Vitamin E exhibited increased sperm concentration and total spermatozoa numbers, had higher percentages of spermatozoa with normal acrosomes and that passed a hypo-osmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities compared to ejaculates from

boars supplemented with inorganic Se (0.2 ppm sodium selenite) and 30 IU vitamin E. However, these results are confounded with level of vitamin E (Jacyno et al., 2002).

The working hypothesis of our laboratory is that because of greater bioavailability, supplementing boar diets with an organic source of Se will lead to superior semen quality and fertility when compared to that of boars fed an equivalent level of inorganic Se or unsupplemented control diets. The objectives of the experiments is to ascertain whether boars fed diets supplemented with 0.3 ppm organic Se would exhibit improved growth performance, carcass characteristics, tissue Se concentrations, testis gene expression, semen characteristics, and semen fertilizing capacity compared to boars fed diets containing an equivalent amount of inorganic Se (sodium selenite) or a basal diet with no additional Se.

CHAPTER 2

LITERATURE REVIEW

I. Overview of breeding systems used in the commercial swine industry

Types of swine breeding systems. There are three breeding methods used in the swine industry: pen mating, hand mating, and AI. Regardless of the method used, the ultimate goal for pork producers is to maximize farrowing rates (number of sows and gilts farrowing/number of sows and gilts exposed to the boar) and the number of piglets born alive. With pen mating, a boar is placed in a pen of females to breed. Lower farrowing rates typically accompany pen mating because sows and gilts are not always bred during periods of optimum fertility because the timing of mating relative to the expected time of ovulation is not controlled. Boars may mate with one female in estrus numerous times, completely ignoring other females in estrus. Another disadvantage with this method is dates of when sows or gilts are bred are unknown, making it difficult to predict farrowing dates. This method of breeding is typically used in small commercial operations because of reduced labor and minimal facility requirements.

When decreases in reproductive performance characteristic of pen mating systems are considered, other mating strategies such as hand mating and AI prove more beneficial despite the increased time and labor associated with these techniques. As such, hand mating and AI are the most common methods used in large commercial operations. With hand mating systems, females are checked for estrus and mating between the boar and the female is observed. Producers can make sure sows and gilts are bred multiple times, maximizing the likelihood that females are mated at the optimum time relative to ovulation (0 to 24 h prior to ovulation; Soede et al., 1995). Unlike pen mating, the producer using hand mating systems is able to determine

the number of females bred, anticipate when they will farrow, and critically evaluate the breeding performance of both the boar and females.

In AI systems the producer inseminates the female with semen that is either collected on the farm or obtained from an outside source. The use of AI offers many benefits over natural mating systems. Artificial insemination allows producers more extensive use of genetically superior sires and gives producers the ability to bring in new genetic material with a decreased risk of sexually transmitted diseases. Commercial boar studs sell semen to producers from a wide variety of breeds and sire lines. As a result, fewer boars are needed on the farm reducing feed, veterinary, and housing costs. Reductions in labor costs are seen with AI programs because breeding sows via AI requires less time compared to hand mating systems (Flowers and Alhusen, 1992). There is also lowered risk of injuries to the sow, the boar, and the producers handling them, when AI is used.

Producers can obtain semen from a number of different sources. First, semen could be collected on the farm. While this method provides a producer with a readily available supply of fresh semen and control of genetic decisions, the cost of the equipment to collect, evaluate and process semen can be high. When collection is done on the farm, producers typically use higher sperm concentrations in each insemination dose, since they tend to use all the semen collected in a day rather than store it. Producers may also mix semen from multiple boars, which may also encourage producers to use more spermatozoa than is needed (Johnson et al., 2000). Semen can be collected at boar "studs" that allow producers to retain ownership of their boars. Producers are charged "room and board" and fees are assessed to collect and process semen. While this method is more expensive on a month-to-month basis, it saves the producer time otherwise used to collect and process semen, as well as money from having to purchase and maintain specialized

equipment. Some producers have banded together to create cooperative boar studs from which they can obtain semen. In addition to broadening the genetic pool compared to what is available at a single farm, this option allows producers to share costs associated with operating boar studs. Semen can also be shipped overnight from commercial boar studs, most of which are located in the Midwest. While this option is usually more expensive than collecting semen on the farm, producers like the option for the obvious time and labor savings of not having to collect semen or do laboratory work. Another plus is the access to a much larger, and often times higher-quality gene pool than to what a producer or a cooperative would have access. Singleton (2000) estimated that 40 to 45% of the semen supply is produced in house by company-owned boar studs, 15 to 20% of the semen supply arises from genetic companies, and 5 to 10% of the semen supply comes from a cooperative, a commercial provider, a fee-for-service provider, or from on-farm sources.

Once semen is collected, the boar ejaculate is diluted with a semen extender, resulting in multiple AI doses that can be used to inseminate sows and gilts. Singleton and Flowers (2007) estimated that one boar can produce 1100 to 1200 doses of semen each yr provided that 22 AI doses are produced each wk. Knox et al. (2008) surveyed North American boar studs and it was reported that boars produce 51 to 150 billion sperm per boar per wk on average, resulting in 21 to 40 doses of extended semen each wk. These average sperm production values equate to boars being able to produce approximately 1092 to 2080 doses of semen each yr. These numbers are similar to those reported by Diehl et al. (1984), who suggested that the average boar could produce enough sperm to impregnate 1200 to 2000 females per yr. These numbers can vary however, because the number of ejaculated sperm can depend on age, collection frequency, and

the length of rest periods between collections. In comparison, in a pen mating system, a boar rarely breeds more than 100 to 200 females per year (Diehl et al., 1984).

Current usage of AI and predicted usage in the future. The use of AI in the U.S. swine industry has increased dramatically over the past two decades. The number of females bred using AI in the U.S. increased from 8% of sows and gilts bred in 1991 (Burke, 2000) to nearly 70% in 2000 (Lawrence and Grimes, 2001). It is believed that the use of AI will continue to increase until practically all commercial pork producers will utilize AI technology. The usage of AI in the U.S. swine industry would then be similar to what is seen in nations such as Norway (Landsverk, 2000), the Netherlands, Denmark, and Spain (Burke, 2000).

Boar semen collection methods. In commercial boar studs, semen from a boar is collected using the gloved-hand technique (Hancock and Hovell, 1959; Singleton, 2001) while a boar mounts an artificial sow. The use of an artificial sow is preferable to using a sow exhibiting estrus when semen is routinely collected from boars. Not only does the artificial sow eliminate problems due to size differences, it also eliminates a sow's reluctance to support a boar's weight until ejaculation has been completed or the tendency of the sow to walk around the pen during collection. While the boar mounts the artificial sow, the spiral end of the boar's penis is grasped by a hand wearing a disposable vinyl glove. Pressure on the distal spiral-shaped end of the penis imitates the sow's cervix and stimulates ejaculation.

Ejaculation consists of three phases, the pre-sperm fraction, sperm rich fraction, and the post-sperm fraction. The pre-sperm fraction has clear seminal fluid, some gel, and has high bacterial counts. This fraction is not kept as it contains relatively few viable spermatozoa. The sperm-rich fraction is easily recognized by the creamy, white appearance. This fraction is kept and has the largest density of spermatozoa. Because this fraction is very sensitive to temperature

changes, semen is collected in prewarmed, insulated thermal containers in order to maintain spermatozoal viability. The post-sperm fraction, that may contribute as much as 250 mL, consists of clear seminal plasma free of spermatozoa, and gel from the bulbourethral glands. Total ejaculation time may take five to 20 min. The gel portion is believed to have little or no physiological importance. It is removed from the ejaculate using gauze, cheesecloth or a mesh filter within the collection bag as the gel coagulates into a mass that interferes with semen evaluation, processing, and AI (Holden and Ensminger, 2006).

Automated collection systems, including Collectis (Genes Diffusion, Madison, WI) and Automate (MiniTube of America, Verona, WI) have been developed over the past decade to decrease labor requirements in large commercial boar studs. In addition to an artificial sow, the Automate system includes an artificial cervix, which provides a firm texture and grip for the boar's penis and a hygienic double sheath plastic liner for the separate collection of the pre-sperm and the sperm rich fractions of ejaculate (Terlouw et al., 2008). The Collectis system regulates air pressure and vacuum to control the volume and pressure of air supplied to an artificial vagina (AV), which holds the boar's penis during collection. Once the boar's penis is inside the AV, a clean filter and collection bag assembly, attached to the AV, provides a hygienic system for ejaculate collection (Aneas et al., 2008).

Terlouw et al. (2008) reported that ejaculate volume, concentration, and motility were similar with the Automate system and the gloved-hand technique. However, there was a 70% reduction in labor for a single collection. Aneas et al. (2008) did not find a difference in semen output with the Collectis system compared to the gloved hand technique, but did report a 90% increase in the number of collections per employee per hour. Lellbach et al. (2008) reported that collection with the Collectis system averaged 12.6 ejaculates collected per hr, while the

Automate system averaged 8.9 ejaculates per hr. In that study, bacterial contamination was lower with the automated collection method compared to the gloved-hand technique and sperm concentration and total spermatozoa were higher for boars collected via the gloved-hand technique compared to the automated methods. Ejaculate volume was highest with the Collectis system, intermediate with the gloved hand technique, and lowest with the Automate system. With the increased price of equipment, AI stations would begin to see a return on investment if at least 7400 semen doses are produced each wk with the AutoMate system, or 9350 semen doses are produced each wk in the case of the Collectis system. However, smaller farms or AI stations producing less than these semen doses would fail to see the benefit of the automated collection methods over the gloved hand technique.

Characteristics of a typical boar ejaculate. Semen characteristics vary among boars, breeds, ages, collection frequencies, resting periods between collections, and a myriad of other environmental and management factors. While boars can ejaculate 50 to 500 mL of semen, ejaculates of approximately 200 mL are more common. Boar ejaculates can contain 20 billion to 200 billion sperm cells (Diehl et al., 1984; McGlone and Pond, 2003). Most recommendations state that sperm motility and morphologically normal spermatozoa should be 80% or better (Diehl et al., 1984). Diehl et al. (1984) states that most boars can have their semen collected one to five times per week. While research has been shown that a minimum of 2 billion live sperm are required to obtain adequate conception rates (Diehl et al., 1984), most semen is packaged in AI tubes or bottles that contain three to five billion spermatozoa in a volume of 70 to 90 mL semen and extender (Colenbrander et al., 2000). Depending on the individual boar and the semen extender used, semen can be stored in an 18°C storage unit for three to seven d and still result in a fertile insemination.

Semen evaluation methods. In order to study the effects of different management strategies on boar fertility, there must be a way to assess fertility of boar semen. Unfortunately, there is no single laboratory test that can be used to accurately determine boar fertility. Although evaluation of sperm motility alone cannot assess the ability of a spermatozoan to penetrate the zona pellucida and fertilize the oocyte in vivo, Tejerina et al. (2008) reported that sperm motility is the parameter that is most frequently used to measure boar sperm viability in the ejaculate during or after storage or cryopreservation.

Classical methods of semen evaluation include determining the volume of ejaculate, spermatozoa concentrations, total spermatozoa in the ejaculate, total motility, progressive motility, the percentage of viable cells, and sperm morphology (Gadea, 2005). Volume of semen can be measured by pouring the semen into a graduated cylinder or by weighing the semen, assuming that the density of semen is one g/mL. Concentration can be measured spectrophotometrically, which measures opacity of the semen, manually using a hemacytometer and a phase contrast microscope, or with a computer-assisted sperm analysis (CASA) system. However, because of the higher number of counted cells and lower variation, the CASA (e.g., Hamilton Thorne semen analyzer, Ceros 12.1; Hamilton-Thorne Research, Beverly, MA) is more reliable than counting chambers when determining sperm concentration. Motility can be subjectively measured using a light microscope or more objectively measured using CASA systems. Vyt et al. (2004b) concluded that because of the high subjectivity associated with visual motility assessments, these methods should be replaced with the more objective CASA system that can also give a more detailed motility assessment. The CASA system has the added advantages of being able to measure spermatozoa exhibiting progressive motility as well as measuring spermatozoal velocity, track characteristics, and head size in a short period of time.

Progressive motility is a measure of both unimpaired metabolism and intactness of the sperm membrane (Johnson et al., 2000). Morphology assessments determine the percentage of spermatozoa with abnormal heads, damaged acrosomal membranes, abnormal tails, bent or coiled tails, heads without tails, or proximal or distal cytoplasmic droplets. To determine morphology manually, a small amount of semen is stained, smeared on a microscope slide, and examined using a light microscope. With the correct software package, CASA can also be used to determine the frequency of morphological abnormalities.

Sutkeviciene et al. (2005) reported moderate correlations between 60-d non-return rate and total motility, as determined by CASA technology for semen extended in X-cell and assessed at d 1 (r^2 = 0.38; $P < 0.05$) and at d 7 (r^2 = 0.54; $P < 0.01$) of storage at 17˚C. Progressive motility was not correlated with the non-return rate 1 d (r^2 = -0.06) and 7 d into storage (r^2 = 0.06). No significant correlations were seen between gilt or sow litter size and total motility or progressive motility. Tardif et al. (1999) reported high correlation (r^2 = 0.78; P = 0.01) between fertility rates and percentage of motile spermatozoa for semen diluted in BTS such that each dose contained 300 million sperm. A second dose with higher concentrations (3 billion sperm) did not exhibit a significant correlation (r^2 = 0.22) between spermatozoa motility and fertility rate. Other CASA measurements or their correlations with fertility were not reported by Tardiff et al. (1999) or Sutkeviciene et al. (2005).

In contrast to these findings, Didion (2008) failed to find significant correlations between any motion parameter evaluated by CASA and matings, defined as the number of females returning to estrus versus the number farrowed, or farrowing rate. During the study females were inseminated with doses containing 3 to 5 billion spermatozoa two or three times per estrus, which is consistent with methods used in the commercial swine industry. The average number of

females bred per boar was 20, which may have not been sufficient to detect statistically significant differences. What is not mentioned is the age of females (sows or gilts or both), whether synchronization protocols were followed, or the timing of inseminations. The author suggested that they might be able to find significant correlations in future studies if they limited sperm concentrations per AI dose, the number of semen doses used throughout estrus, or increased the number of females bred per boar.

Farrell et al. (1998) correlated bull fertility, defined as 59-d nonreturn rates corrected for cow and herd effects, with different CASA measurements. The percentage of motile spermatozoa was positively correlated ($r^2 = 0.34$; $P < 0.05$) with bull fertility. Higher correlation values ($r^2 = 0.68$ to 0.98) were obtained using multiple regression with a mixture of two to five CASA parameters included. For example, fertility was moderately correlated ($r^2 = 0.68$; $P < 0.05$) when amplitude of linear head displacement (ALH) and progressive motility were considered in the model. Fertility was strongly correlated ($r^2 = 0.98$; $P < 0.05$) when ALH, beat cross frequency (BCF), linearity (LIN), average path velocity (VAP), and straight line velocity (VSL) were included in the model.

In vitro fertilization (IVF) is another test used to assess overall sperm function. Harrison (1997) and Gadea (2005) identified binding and penetration of the zona pellucida of oocytes as one of the biggest barriers spermatozoa must pass during fertilization. As such, oocyte penetration rates are the most commonly used measure of boar fertilization capacity. Interactions between the spermatozoa and the oocyte plasma membrane could explain variability of fertilization capacity seen within fertile boars.

Ruiz-Sanchez et al. (2006) evaluated sperm motility assessment and IVF as predictors of relative boar fertility. When bred to approximately 50 gilts using a low number of sperm (1.5

billion sperm), nine boars differed consistently for pregnancy rate (72% to 98%), farrowing rate (71% to 98%), and total born (8.4 to 12.0 pigs). When IVF was conducted, the same nine boars had 94.2% average zona pellucida penetration rate (85% to 99%) and 77.1% oocyte penetration rate (49% to 97%) on at least nine different occasions. Of penetrated oocytes, 39.5% had one male pronucleus (MPN; 22.7% to 54.8%) and 29.7% had more than one MPN (11% to 54%). Average potential embryo production rate, defined as the percent of penetrated oocytes with both a female pronucleus and a single MPN, was 22.8% (1% to 43%). A multiple linear regression model based on sperm motility at d 7 and presence of cytoplasmic droplets accounted for 27% and 22% of the variation of fertility index, defined as the number of total piglets born divided by the number of gilts initially bred per boar, and total pigs born, respectively, whereas male pronuclear formation was the IVF variable that accounted for 17% and 12% of the variation in farrowing rate and fertility index, respectively. When conducting independent linear regressions, correlations ($P < 0.05$) were found between average oocytes penetrated and total pigs born ($r^2 = 0.28$), and average MPN and farrowing rate ($r^2 = 0.26$), total born ($r^2 = 0.31$), and fertility index ($r^2 = 0.31$). The number of oocytes with at least 1 MPN formed was correlated ($P < 0.05$) with pregnancy rate ($r^2 = 0.41$), farrowing rate ($r^2 = 0.41$), and fertility index ($r^2 = 0.35$). Oocyte number with more than one MPN was correlated ($P < 0.05$) with pregnancy rate ($r = 0.26$), farrowing rate ($r = 0.28$), total born ($r = 0.25$), and fertility index ($r = 0.30$). Because sperm motility at d 7 and presence of cytoplasmic droplets accounted for a larger amount of variation in fertility index compared to MPN formation, the authors concluded that sperm motility was still a better tool at predicting boar fertility compared to IVF parameters.

Gil et al. (2008) considered IVF a sub-optimal tool for predicting boar fertility, due to the incidence of oocyte polyspermy, as well as both inter- and intra-boar variability in sperm

characteristics. Instead of using IVF to predict fertility of all boars within a commercial boar stud, Ruiz-Sanchez et al. (2006) suggested that IVF could be useful to screen infertile boars or boars of questionable fertility that existed in boar studs. This is in part due to the expense of IVF. In addition to the expense of purchasing an incubator that can hold oocytes at 39˚C in a 5% CO_2 environment under high humidity, aspirating oocytes from ovaries, and incubating them in the correct media prior to, during, and after fertilization is very tedious and time consuming. It is possible to purchase oocytes from a commercial source to save time, but this time savings would have to be weighed against the expense of the oocytes.

Semen Preservation. There are two methods to preserve boar semen: frozen or liquid semen stored at 16 to 18 °C for up to 10 d. Freezing boar semen offers long-term storage of important genetic material and eliminates the need of transporting animals or fresh semen over long distances or extended periods of time. Frozen semen could be used to repopulate breeding herds after the outbreak of a contagious disease (e.g., porcine circovirus type 2 or porcine reproductive and respiratory syndrome) or if a natural disaster occurred (e.g., a fire). Compared to fresh semen, a greater number of spermatozoa are needed per dose of frozen semen because 40 to 50% of the spermatozoa do not survive the freezing process (Bailey, 2008). Low spermatozoal survivability is also associated with low fertilization rates, and smaller litter sizes (Gilmore et al., 1998). Injuries to spermatozoa incurred during freezing and thawing include cold shock, osmotic stress, and intracellular ice crystal formation leading to a large number of spermatozoa dead or grossly damaged. If spermatozoa are able to survive, shortened life spans are common (Bailey et al., 2000).

Johnson et al. (2000) reported that less than 1% of all sow and gilt inseminations worldwide are made using frozen-thawed semen, with insemination doses exported from one

country to another for the purpose of upgrading the genetic base of a particular country or herd. Cordova et al. (1997) found average in vitro fertilization rates were 68% with frozen-thawed spermatozoa, compared to 85% for fresh semen. While the theoretical advantages of cryopreservation are inarguable, the many disadvantages have resulted in commercial breeding programs preferring liquid storage methods that do not negatively affect the spermatozoa.

There is ongoing research to improve sperm survival after cryopreservation. Maldjian et al. (2005) reported general decreases in polyunsaturated fatty acids and significant reductions in cholesterol in spermatozoa after cryopreservation. Increasing the amount of omega-3 fatty acids, by using egg yolks enriched in docosahexanoic acid in the diluent, failed to improve sperm quality after cryopreservation. Bailey et al. (2008) hypothesized that since the sperm plasma membrane is the primary site of freezing-induced damage, reinforcing the membranes with cholesterol would improve the ability of boar spermatozoa to withstand cold temperatures associated with cryopreservation. Cyclodextrins, cyclic oligomers of glucose that form water soluble complexes with other non-water-soluble organic molecules can be used to deliver cholesterol to the sperm plasma membrane. Although there were no differences in post-thaw motility or progressive motility of sperm, Bailey et. al (2008) discovered that cholesterol-loaded methyl-β-cyclodextrins improved sperm viability in BF5 diluent without egg yolk for 0 and 30 min post thaw. This method of cholesterol delivery has been previously used to improve the cryosurvival of ovine and bovine sperm, although in vivo and in vitro fertility was unaffected (Bailey et al., 2003).

Additional experimentation has been aimed at alleviating the impact of lipid peroxidation, which boar spermatozoa are exposed to during freezing and thawing, and which damages sperm membranes and impairs energy metabolism. According to a review by Grossfeld

et al. (2008), the addition of antioxidants or chelating agents (e.g., catalase, vitamin E, glutathione, butylated hydroxytoluene, or superoxide dismutase) to the standard egg yolk-based diluents prevented the damage observed during the freezing and thawing of spermatozoa. Other management approaches used to improve the viability and fertility of frozen-thawed spermatozoa include individually optimizing cooling and thawing procedures for sperm cells collected from boars that are sub-standard with regard to the ability of their gametes to survive cryopreservation, deep intrauterine insemination, and minimizing insemination-to-ovulation intervals.

According to Johnson et al. (2000), there are two main factors affecting sperm cell function after ejaculation and during storage: the conditions of the diluent, and the temperature at which the semen is collected and stored after dilution. A vast number of diluents have been developed and used to preserve the lifespan of spermatozoa during liquid storage. While the formulations of the semen extenders differ, they all serve as a media to prolong spermatozoa survival and maintain their capacity to fertilize oocytes. Semen extenders increase the total volume of diluted semen, provide spermatozoa with nutrients for metabolism, protection from rapid cooling, shifts in pH, and changes in osmotic pressure, and provide antibiotics to inhibit bacterial growth (Holden and Ensminger, 2006).

Liquid semen extenders can be divided into three groups, short-term extenders, those that maintain spermatozoa for up to three d, mid-term extenders that maintain spermatozoa for five to seven d, and long-term extenders that maintain spermatozoa for as many as seven to 10 d. Beltsville Thawing Solution (BTS) and the Kiev extender are examples of short-term extenders. Sperm Aid, MR-A, X-Cell, Modena, Androhep-Lite, and Vital are examples of mid-term extenders. Androhep Enduraguard is an example of a long-term semen extender (Estienne et al.,

2007). A recent survey of boar stud practices by Knox et al. (2008) ranked the use of semen extenders as follows: Androhep, X-Cell, BTS, Androhep Enduraguard, Geddil, Preserv, Ivo-Zeist and MR-A, and finally Acromax, Cell-Lution, Safe-Cell, Survivor, and Vital. The marketing of the newer semen extenders is very competitive, and therefore exact chemical compositions of the extenders such as Androhep, Androhep-Plus, Androhep Enduraguard, Acromax, X-Cell, MR-A, V.S.P., and Vital are tightly held proprietary secrets (Levis, 2000).

Long-term extenders are beneficial when sending semen long distances or when time is needed to inspect quality of the semen. Even with the development of long-term extenders, however, the majority of producers still inseminate gilts and sows on the first few days following collection (Gerrits et al., 2005). According to Levis (2000), producers are using long-term extenders as short- and midterm-extenders because they have been told the long-term extenders are a more superior product. Even though the long-term extenders are more expensive, producers do not want to risk potential decreases in reproductive performance.

Boar spermatozoa are very susceptible to cold shock, which occurs when freshly ejaculated boar spermatozoa are cooled quickly from body temperature (38˚C) to temperatures below 15˚C, and results in the loss of sperm motility and viability. Zou and Yang (2000) examined the effect of storage temperature (4˚, 15˚, 20˚, and 39˚C) on fresh, non-extended semen over a 48-h period. After 48-h of storage, viability of spermatozoa, as determined by trypan blue staining was 1.6%, 46.9%, 42.0%, and 31.0% for 48-h storage at 39˚, 20˚, 15˚, and 4˚C, respectively. The hypoosmotic swelling test measures how many sperm have normal acrosomal membranes by incubating sperm in a hypoosmotic solution; normal sperm heads swell and the sperm have coiled tails, whereas abnormal sperm do not exhibit swelling of the head or curling of the tail. After 48-h storage, 1.7%, 28.7%, 24.1%, and 20.1% of spermatozoa exhibited coiled

tails for 39˚, 20˚, 15˚, and 4˚C storage temperatures, respectively. With coomassie blue staining, which measures acrosome-intact spermatozoa, 4.5%, 35.3%, 55.7%, and 22.8% of the spermatozoa exhibited intact acrosomes after 48-h storage at 39˚, 20˚, 15˚, and 4˚C, respectively. Fluorescein isothiocyanate-peanut agglutinin measures the ability of sperm to complete the acrosome reaction. After staining with this dye, 4.3%, 43.2%, 17.3%, and 14.8% of spermatozoa would have been able to complete capacitation after 48-h storage at 39˚, 20˚, 15˚, and 4˚C, respectively. Sperm motility was positively correlated with each of the tests conducted to measure sperm viability: trypan blue staining (r^2 = 0.94), hypoosmotic swelling test (r^2 = 0.95), coomassie blue staining (r^2 = 0.90), and fluorescein isothiocyanate-peanut agglutinin (r^2 = 0.86). The authors concluded that sperm can be stored at 15˚ or 20˚C without harming membrane integrity or viability.

Althouse et al. (1998) attempted to find the lower critical temperature and time at which extended boar semen underwent cold shock by incubating semen extended with Androhep at different temperatures. Sperm motility dropped below 70% within 12-h of exposing semen to 8˚C and within 48-h of exposing semen to 10˚C temperatures. Sperm motility was not adversely affected by temperatures of 12˚, 14˚, and 17˚C within the 48-h experiment. Storing extended semen at 12˚ or 17˚C did not adversely affect farrowing rates (93% and 95%, respectively), total offspring born (11.58 and 11.61, respectively), or number of piglets born live (10.68 and 10.63, respectively). .

Summary and implications for future research. The use of AI has dramatically increased over the past two decades in the U.S. commercial swine industry. Capturing the maximum benefits of AI necessitates having boars that are easily trained to consistently mount an artificial sow and allow semen collection, and that ejaculate semen containing large numbers

of fertile sperm cells on a consistent basis. Unfortunately, little information exists regarding management techniques that optimize semen quality and sexual behavior in boars used for AI. One potential strategy for enhancing semen quality is to feed AI boars diets supplemented with trace minerals such as Se. New information suggests that the form of the minerals supplemented, i.e., Se from an organic source versus Se from an inorganic source, is very important. Feeding the organic source of Se, in the form of selenomethionine, compared to an inorganic form of Se, such as sodium selenite, could result in improvements in boar reproductive performance.

II. Importance of selenium in animal diets

Selenium is a trace mineral that was discovered in 1818 by Berzelius in Sweden. No biological significance was associated with the mineral until it was identified as a toxic agent involved with lameness and death in livestock grazing certain plants in Wyoming and the Dakotas as early as 1857 (Franke, 1934). The establishment of Se as an essential nutrient (Schwarz and Foltz, 1957) led to a new era of research that is still ongoing. In 1973, Se was shown to be a component of the antioxidant enzyme glutathione peroxidase (Rotruck et al., 1973). Despite the significance of this finding, it is probable that this is not the only metabolic role that Se fulfills.

Free radicals. As reviewed by Halliwell and Gutteridge (2000), free radicals are atoms or molecules containing one or more unpaired electrons that are often derived from molecular oxygen or nitrogen. Free radicals are highly unstable, reactive, and are capable of damaging many biologically relevant molecules such as DNA, RNA, proteins, lipids, or carbohydrates. Free radicals can be produced as a consequence of normal metabolic activity or as part of the immune system's strategy for destroying invading microorganisms. Internal sources of free

radicals include mitochondria and the electron transport chain, phagocytes, xanthine oxidase, reactions with Fe^{2+} and Cu^{+}, arachidonate pathways, peroxisomes, inflammation, and biomolecule oxidation (epinephrine, dopamine, tetrahydrofolates, etc.). External sources can include cigarette smoke, radiation, UV light, pollution, certain drugs, chemical reagents, and industrial solvents. The collective terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been introduced and include not only the oxygen or nitrogen radicals, but also some non-radical derivatives of oxygen and nitrogen. These include the radicals alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), and nitrogen dioxide ($NO_2^{\bullet}$). Important nonradicals classified as ROS and RNS include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), nitroxyl anion (NO^-), and nitrous acid (HNO_2; Halliwell and Gutteridge, 2000).

As reviewed by Surai (2006), superoxide is the main free radical produced in biological systems and can inactivate some enzymes due to formation of unstable complexes with transition metals. Superoxide has a half-life of $1x10^{-6}$ s and can act as either an oxidizing or reducing agent. Superoxide itself is not extremely dangerous due to the fact that it cannot rapidly cross the lipid membrane bilayer, but the danger is that it is the major precursor of other, more powerful ROS. For example, superoxide can react with nitric oxide to create peroxynitrite, a very potent oxidant. The hydroxyl radical, on the other hand, has a short half-life ($1x10^{-9}$ s), but is the most powerful radical in biological systems. The hydroxyl molecule can damage any molecule it touches but the damaging effect of the hydroxyl radical is restricted to the site of its formation. Hydrogen peroxide has a longer half-life and is not as reactive as the hydroxyl molecule, but the hydroxyl molecule can be generated when hydrogen peroxide is in the

presence of Fe^{3+} via the Fenton reaction. Nitric oxide is a short-lived molecule that regulates many physiologic functions by itself, some of which are associated with development. According to Turner and Lysiak (2008), a longer half life can result in the potential for a greater diffusion distance, which can allow reactive species to damage more remotely from their source of formation.

As reviewed by Szabo et al. (2007), reactive oxygen species can lead to a host of physiological problems. Reactive species can inhibit antioxidant enzymes, which can result in the diminished ability of the cell to protect itself from oxidant and free radical damage. Depletion of antioxidant enzymes has been reported in response to reactive species, which also reduces the ability of the cell to protect itself from oxidant damage. They can also inhibit various other cytosolic enzymes, leading to general cell function impairment. Reactive species can inhibit membrane channels, which can change cellular ionic balance and cellular calcium handling. Imbalances of intracellular calcium can also lead to cell energy depletion, enzyme dysfunction, and promotion of cell death. Reactive species can also lead to aggregation of proteins, including Lewy bodies, which are spherical bodies that aggregate in nerve cells, displacing other cell components, that are associated with diseases including Parkinson's disease. Impairments due to reactive species have been noted in tetrahydrobiopterin (BH4)-mediated enzymes as well as NAD-dependent enzymes. Reactive species can also cause dysregulation of cell adhesion molecules, which can result in an enhanced inflammatory response. Lipid peroxidation and nitration can lead to oxidation and nitration of fatty acids and thiol modification of proteins, all of which destabilizes cell membranes. Mitochondrial dysfunction due to reactive species can result in the release of mitochondrial death factors and generation of mitochondrial oxidants and free radicals. Reactive species can also have a genotoxic effect, damaging DNA

and leading to a host of problems including DNA mutations, which may be passed along to future generations, and could cause cancer and cell death (Szabo et al., 2007).

Antioxidant defenses. As reviewed by Surai (2000, 2006), there are three lines of antioxidant defense in animal cells. The first line of defense serves to prevent radical formation by removing precursors of free radicals or by inactivating catalysts. The first line of defense includes enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and metal-binding proteins. Since the superoxide radical is the main free radical produced in cell physiological conditions, superoxide dismutase is considered to be the main element of the first line of defense. Superoxide dismutase catalyzes the dismutation of the superoxide radical into hydrogen peroxide and oxygen. Hydrogen peroxide can then be detoxified by glutathione peroxidase (GPx) (see below) or catalase, both of which reduce the peroxide to water (Surai, 2000, 2006).

The first line of defense is not sufficient to completely prevent free radical formation. Chain-breaking antioxidants such as vitamin E, ubiquinol, carotenoids, vitamin A, ascorbic acid, uric acid, and GPx are members of the second line of defense. Vitamin E has been shown to be the most effective natural free radical scavenger to date and is the main chain-breaking antioxidant in the cell. Chain-breaking antioxidants inhibit peroxidation by scavenging peroxyl radical intermediates and keeping the chain length of the propagation reaction as small as possible. However, hydroperoxides, which are produced in the reaction of vitamin E and the peroxyl radical, are toxic and if not removed, can impair membrane structures and functions. Glutathione peroxidase, a Se-dependent enzyme, is the only enzyme able to convert hydroperoxides into nonreactive products. As such, vitamin E only completes half of the job,

and must work equally and in tandem with GPx in order to scavenge free radicals (Surai, 2000, 2006).

Because the second level of defense is not able to deal with the damaging effects of ROS and RNS on lipids, proteins, and DNA, a third level of antioxidant defense enzyme includes lipases, peptidases, proteases, transferases, and DNA repair enzymes to excise and repair the damaged portions of molecules. Since maintaining genome integrity is of vital importance, humans and animals have evolved a range of systems that can recognize, signal the presence of, and repair the various forms of DNA damage. DNA repair mechanisms include the direct reversal of the lesion, mismatch repair, base excision repair, nucleotide excision and incision repair, transcription-coupled repair, global genome repair, translesion synthesis, homologous recombination, and nonhomologous end-joining. These repair pathways are universally present in living cells and are extremely well conserved genome to genome (Surai, 2000, 2006).

Oxidative stress, caused by the production of reactive substances, plays a role in a number of conditions known to be detrimental to human male fertility (Aitken, 1994; Sanocka and Kurpisz, 2004; Turner and Lysiak, 2008). A review by Aitken (1994) explained that one of the most significant factors in human male infertility was a loss of sperm function as a consequence of oxidative stress. The plasma membrane of mammalian spermatozoa, unlike mammalian somatic cells, contains very high levels of phospholipids, sterols, and saturated and polyunsaturated fatty acids (PUFA), all of which are very susceptible to ROS damage (Jones et al., 1979; Sanocka and Kurpisz, 2004). The peroxidation of PUFA in the sperm plasma membrane leads to a loss of membrane fluidity, which causes a loss of motility and fertilizing potential, and the induction of DNA damage within the nuclei of spermatozoa (Sanocka and Kurpisz, 2004). Sanocka and Kurpisz (2004) reported that all the major antioxidant systems are

present in human semen, including superoxide dismutase, catalase, GPx, vitamin E, β-carotene, ascorbate, urate, and the transition-metal chelators transferrin, lactoferrin, and caeruloplasmin. However it is likely, as Aitken (1994) suggested that these antioxidant systems, while present, are limited in their effectiveness because of their distribution and concentrations.

Spermatogenesis itself produces ROS, largely from mitochondrial respiration and the catalytic reactions of the steroidogenic cytochrome P450 enzymes. If the production of ROS during spermatogenesis is uncontrolled, the ROS produced damages mitochondrial membranes and can contribute to the inhibition of subsequent steroid production (Luo et al., 2006). Furthermore, Aitken and Clarkson (1987) indicated that human spermatozoa have the ability to generate ROS but do not possess the ability to repair the spermatozoon membrane if damaged by ROS. According to Aitken (1994), spermatozoa are highly dependent on the antioxidant properties of seminal plasma because their own capacity to withstand oxidative stress is so limited. Chabory et al. (2010) attributes this to the fact that fully mature haploid spermatozoa are largely devoid of cytoplasm and are as a result poorly equipped to fight free radical attack and completely reliant on their environment for antioxidant defenses. In fact all of the antioxidant systems within human spermatozoa have been isolated to the midpiece of the spermatozoan, and as such are not able to protect the plasma membrane along the majority of the cell (Aitken, 1994). These ROS attack PUFA within the sperm membrane, which confer fluidity to the plasma membrane allowing it to participate in sperm-oocyte membrane fusion events that take place during fertilization (Aitken, 1994).

A review by Chabory et al. (2010) highlighted the duality of ROS on sperm fertilizing capacity. While spermatozoa are quite sensitive to ROS attack, the ROS that spermatozoa produce themselves, primarily ($\bullet O2^-$ and H_2O_2) have been found to be key players in signal

transduction pathways that lead to sperm capacitation, a key maturation step after ejaculation that must occur in the female reproductive tract *in vivo* preceding fertilization. In contrast, leukocyte contaminated semen samples can produce ROS that can have a negative impact on sperm fertilizing capability, and is associated with human subfertility or infertility (Aitken, 1994). If insufficient ROS are produced, sperm cells will not mature, whereas if too much ROS are produced, spermatozoa will incur oxidative damage rapidly. As such, antioxidant protection of spermatozoa during sperm maturation and storage is very important.

While data comparing boar and human spermatozoa susceptibility to ROS is not found, there has been some research highlighting the differences between phospholipid composition of boar and human sperm plasma membranes. Lessig et al. (2004) reported lower variability phospholipid and fatty acid composition of spermatozoa from boars compared to human spermatozoa. Compared to humans, boar spermatozoa contain much higher moieties of alkyl-linked compounds as well as phosphatydylethethaloamine. Ahluwalia and Holman (1969) found that sperm tails were particularly rich in PUFA, with C_{22} ω-3 and ω-6 fatty acid contents of 10% in humans and 40% in boar sperm tails. It is unknown whether these differences in lipid composition in boar and human sperm plasma membranes could lead to differences in how these species' spermatozoa respond to ROS. Lipid peroxidation within the sperm membrane could be one mechanism responsible for the physiological and biochemical changes seen in spermatozoa during sperm storage (Cerolini et al., 2000).

Furthermore, data on the changes of boar spermatozoa lipid composition and their susceptibility to peroxidation during semen storage is limited. One method that has been adopted to combat peroxidation throughout liquid semen storage is the addition of antioxidants

to semen extenders. Another possible method to combat spermatozoa peroxidation throughout storage is the inclusion of dietary antioxidants, such as Se.

Selenium distribution. Although Se is widely distributed in minute amounts throughout the earth's crust, concentrations and availability in soil vary based on region. The Se content of most soils ranges between 0.1 and 2.0 ppm, but can be as high as 10 ppm in areas known to have soils high in Se (Jackson, 1964). However, only a portion of the Se in soils is available to the vegetation they support. Regions in the U.S. where Se levels are low include the Pacific Northwest (including Northern California), the Great Lakes states, the Northeast, the Atlantic seaboard, and Florida. It is when feedstuffs are grown in these areas that Se supplementation is required to ensure animals stay healthy and exhibit maximum growth and reproductive performance (NRC, 1983).

Selenium deficiency and toxicosis. Signs of Se deficiency are identical to those of vitamin E deficiency and cannot be separated from one another because of their common biological functions, even though the two nutrients function at different locations within the cell (Mahan, 2001). Signs of Se deficiency can include edema (exudative diathesis), hepatic necrosis, edema of the spiral colon, lungs, subcutaneous tissues, and submucosa of the stomach, bilateral paleness and dystrophy of skeletal muscles (white muscle disease), mottling and dystrophy of the myocardium (mulberry heart disease), impaired reproduction, reduced milk production, impaired immune response, and sudden death (Mahan, 2001; Surai, 2006). While reduced prothrombin time, increased red blood cell hemolysis, morphological abnormalities of bone marrow cells, and a decrease in oxygen content in red blood cells that can cause labored breathing occur in stressed Se or vitamin E deficient pigs, anemia is not always seen (Mahan, 2001).

Clinical Se deficiency is rarely seen in the commercial swine industry. Subclinical Se deficiency could be responsible for reduced growth and reproductive performance, but the true impact of subclinical Se deficiency is unknown (Surai, 2006; Close et al., 2008). Prior to the approval of Se supplementation in animal diets, annual reported losses associated with Se deficiency cost the beef cattle, dairy cattle, and sheep industries nearly $545 million in 1975 (Ullrey, 1980). Subclinical Se deficiency affected some swine herds such that mortality in growing pigs reached 15 to 20%, while morbidity reached 25% or more. The inability to supplement swine and poultry diets with selenium led to losses of $82 million. Furthermore, reductions in reproductive efficiency and resistance to environmental stressors and disease were also reported in the swine industry. Similar death losses and productivity declines were also seen in poultry and other livestock species (Ullrey, 1992).

According to Maiorino et al. (1999), Se deficiency appears to affect reproduction in males to a greater extent than females. Unlike other Se deficiency syndromes, male infertility is not a phenomenon commonly observed in man and animals, since it only develops after sustained Se deprivation. The reason for the delayed onset is likely due to the still unexplained fact that the testis uses dietary Se from the gastrointestinal tract to maintain Se status preferentially over other tissues, such that it is rarely critically depleted under natural conditions (Behne et al., 1982; Maiorino et al., 1999). As a result, experiments with Se-deficient mice are usually completed with second- or third- generation of Se-depleted mice (Behne et al., 1996). Similar studies with second- or third- generation Se-depleted pigs have not been reported.

Signs of clinical and subclinical Se deficiency are highly responsive to Se supplementation (Behne et al., 1996). Besides supplementing diets with Se, other ways of ameliorating signs of Se deficiency are available. Pehrson (1993) stated that it is not uncommon

in practice to give single or repeated injections of Se (with or without vitamin E) for prevention of specific cattle herd problems, such as retained placenta in dairy cattle. Pehrson (1993) questions this practice, however, because selenium can be given easily as a feed additive and the animals would not have to be stressed by giving them unnecessary injections. There are some exceptions however, such as cattle and sheep that are out on pasture. In these cases, producers can either administer a single injection of a slow-releasing selenium or they can administer a slow-releasing trace element capsule that is deposited into the rumen (Pehrson, 1993).

A review by Pehrson (1993) identified several ways that producers can increase the selenium content of feedstuffs. Spraying a single application of a liquefied sodium selenite onto crops can lead to the transformation of inorganic selenium to organic selenium in plants within a few hours. Foliar application is not easy for crops that are harvested more than once per year, and spraying poisonous sodium selenite is hazardous to human health. Including selenate or selenite into fertilizers has been shown to increase Se levels in grains (Pehrson, 1993). However, selenate is more effectively absorbed by plants compared to selenite. Including Se-fertilized grains in animal diets can then improve the Se status of animals. There is however a great deal of variation in the uptake of selenium from selenate between overwintering and spring-sown crops, and also between first and second cuttings of grasses (Pehrson, 1993). According to Pehrson (1993), New Zealand and Finland are two Se-deficient countries that have added selenate to fertilizers to both treat Se deficiency in animals and increase the average Se intake of people. Continued application of selenized fertilizer has been frowned upon by environmentalists even though no problems have been reported as a result of fertilizing the soil in those countries where this practice has been followed (NRC, 1983).

Signs of Se toxicity include anorexia, hair loss, fatty infiltration of the liver, degenerative changes in the liver and kidney, edema, occasional separation of the hoof and skin at the coronary band, and symmetrical, focal areas of vacuolation and neuronal necrosis. Signs of Se toxicity have been reported for swine consuming diets containing 5 ppm Se or higher (NRC, 1998).

Indicators of selenium status. In order to measure whether an animal is receiving a diet that is deficient, adequate, or toxic in Se, Se status must be evaluated. Concentrations of Se in animal tissues vary with the amount and form of dietary Se supplementation, and the history of Se intake. There is also a substantial amount of variability between animals within a given tissue. Selenium concentrations within plasma, serum, or whole blood are related to Se intake, all raise congruently with each other, and can vary with the form of Se fed. Tissue Se concentrations in cattle, sheep, and swine rank as follows: kidney > liver > testes > heart > skeletal muscle > adipose tissue (Ullrey, 1987). Due to differences in retention, tissue Se concentrations are usually greatest when an organic selenized yeast is fed compared to the inorganic sodium selenite (Kim and Mahan, 2001a,b). Selenium-dependent GPx activities, which are positively correlated with blood Se, can also be used to measure Se status in blood and tissues (Ullrey, 1987). In addition to having numerous members of the GPx family, there are key differences in how GPx enzyme activity is determined. For instance, enzyme activity of cytosolic glutathione peroxidase (GPx1) is determined using hydrogen peroxide, whereas enzyme activity of phospholipid hydroperoxide glutathione peroxidase (GPx4) is determined using phosphatidylcholine hydroperoxide as a catalyst (Lei et al., 1997).

While hair concentration is not always a good indicator of status of all minerals, Combs et al. (1982) and Combs (1987) reported that the content of Se in hair is correlated with Se

dietary intake. Kim and Mahan (2001b) reported that hair color influenced Se levels within hair, with light haired pigs, including both white and red haired pigs, having higher hair Se concentrations compared to black-haired pigs. Correlations between dietary Se intake and hair Se content was greater for organic Se-fed light and dark haired pigs ($r^2 = 0.95$ and $r^2 = 0.97$, respectively) than inorganic Se-fed light and dark haired pigs ($r^2 = 0.80$ and $r^2 = 0.90$, respectively). Ullrey (1987) concluded that milk Se concentrations are influenced by dietary Se. Other tissue Se concentrations may be more reliable indicators of Se status as the concentrations of Se in milk are lower than what is found in other tissues, even when expressed on a DM basis.

A review by Thomson (1998) noted that the Se content of tissues is highly dependent on the form of Se ingested. Because selenomethionine is metabolized the same way that methionine is, it is incorporated into body tissues in a non-specific and unregulated manner, whereas selenite and other forms of selenium are incorporated into a pool of Se sources that the body can incorporate into other proteins, which are regulated by homeostatic mechanisms. When selenomethionine is consumed, it is retained in tissues and tissue proteins to a greater extent than other sources of Se, for which excess is excreted in the urine. As such, one cannot depend on tissue concentrations of Se for the low or high levels of biologically active selenoproteins.

Selenium Requirement of Pigs. The National Research Council (1998) developed Se requirements for growing pigs: 0.3 ppm for 3 to 10 kg pigs, 0.25 ppm for 10 to 20 kg pigs, and 0.15 ppm for 20 to 120 kg pigs. The Se requirements for gestating sows, lactating sows, and sexually active boars were set at 0.15 ppm Se. Expressed in total requirement per day, growing pigs weighing two to five kg require 0.08 mg/d, five to 10 kg BW require 0.15 mg/d, 10 to 20 kg BW require 0.25 mg/d, 20 to 50 kg BW require 0.28 mg/d, 50 to 80 kg BW require 0.39 mg/d, and 80 to 120 kg pigs require 0.46 mg/d. Gestating sows require 0.3 mg/d, whereas lactating

sows require 0.8 mg/d. Sexually active boars require 0.3 mg/d. However. research has shown, as will be discussed later, that increasing the dietary inclusion rate to 0.3 ppm Se will improve performance of sows and boars.

Stress, caused by environment, management practices, and technological factors, and pigs' living conditions within the commercial industry can all influence the selenium requirements of pigs. Compared to pigs raised in commercial conditions, pigs raised under experimental conditions are often less stressed (Close et al., 2008). As such, one could hypothesize that heat-stressed pigs would have a greater Se requirement compared to pigs raised in conditions with lower temperatures and humidity levels. It would also seem logical that pigs in a "clean" environment would have lower Se requirements, although no studies have been conducted to test this hypothesis.

Current FDA regulations. In 1974, the U.S. FDA first approved the use of Se supplements in the form of sodium selenite or sodium selenate for supplementation of swine and growing chicken diets at 0.1 ppm, and at 0.2 ppm for turkeys. In 1982, the FDA approved the addition of up to 0.3 ppm of Se to diets for pigs up to 20 kg because the addition of 0.1 ppm Se did not always prevent signs of Se deficiency in weanling pigs. The FDA later updated this legislation to allow Se supplementation up to 0.3 ppm in diets for chickens, turkeys, ducks, swine, sheep, and cattle (FDA, 1987). In 2000, FDA amended their regulations once more to allow the addition of an organic Se source, selenized yeast (Sel-Plex; Alltech , Nicholasville, KY) to the diets of chickens. The regulations were later amended for the addition of up to 0.3 ppm as selenized yeast to swine and turkey diets in 2002, and for cattle diets in 2003 (FDA, 2003).

The NRC (1983) does recognize that biological availability is very important with Se sources but does not adjust requirements based on the source that would be used in a diet. They do mention, however, that the required concentration of selenium in the diet may need to be as much as twice as great in a situation in which bioavailability is only 50 percent that of a more useful Se source. Rough estimates of Se content of feedstuffs are available (NRC, 1983) without mention of differences of Se content due to Se content within the soil. No additional information is given to guide nutritionists or producers on how to estimate bioavailability of Se(NRC, 1983), and this was before the organic Se supplements were approved, which can vary greatly product to product (Rayman, 2004). As of yet, no distinction has been made between inorganic and organic Se concerning nutrient requirements, which is curious, as the different sources of Se have differing availability, biological activity, digestibility, and metabolic pathways just within one single species (Pehrson, 1993).

Environmental concerns with selenium supplementation. As reviewed by Ullrey (1992), a number of organizations have argued that the FDA failed to address environmental impacts prior to approving the increased Se supplementation level of 0.3 ppm. The primary concerns were over the bioaccumulation of Se in water and its effects on aquatic birds. High Se levels were blamed for the death and deformities seen in aquatic birds and other organisms in the Kesterson Reservoir in Fresno County, CA. Reports surfaced blaming the increased Se levels on prehistoric rocks that line ridges along the west side of the valley instead of Se entering the environment from the legal use of Se supplementation in animal diets. In fact, total feed and veterinary uses of Se would account for less that 8.8% of the total industrial uses of Se. To express this in a different manner, if all the Se from animal feeds were to enter the environment,

it would account for no more than 0.5% of the Se that originated from natural and man made sources (Ullrey, 1992).

Selenium sources. In nature, Se exists in two chemical forms, inorganic and organic. Elemental forms can be reduced to the Se^{2-} oxidation state (selenide) or oxidized to Se^{4+} (SO_3^{2-}, selenite) or Se^{6+} (SO_4^{2-}, selenate). As a result, inorganic Se can be found in the different inorganic forms of selenide, selenite, and selenate. Plants absorb Se from the soil in the form of selenite or selenate and synthesize organic Se-containing amino acids, such as selenomethionine (SeMet) and selenocysteine (SeCys) that exist in the Se^{2-} oxidation state. Naturally occurring organic Se is represented as a mixture of Se-containing amino acids with SeMet representing 50% of the total Se in animal feed ingredients, including cereal grains and roughages (Olson and Palmer, 1976; Combs and Combs, 1986).

As reviewed by Mahan (2001), in well aerated alkaline soils, Se exists as selenite or selenate. Selenate is highly absorbed by plants, followed by selenite, while selenide is not very well absorbed. Sandy soils do not contain much Se because minerals can easily leach out. Adding limestone to soils increases soil pH, which stimulates the conversion of the reduced form (i.e., selenide) to the oxidized forms (i.e., selenate and selenite). The oxidized forms can complex with other minerals in the soil, which can affect its availability to plants. When the oxidized form complexes with iron, it converts the oxidized form of Se to a reduced form of Se. Sulfur can compete with selenium at the root membrane, affecting the amount of Se absorbed by plants. Because of all of these potential issues, it is available Se, not total Se content in the soil that is important to plants (Mahan, 2001).

Until recently, supplemental Se in animal diets had been primarily in the form of inorganic Se, as sodium selenite. The main reason for the preference of adding inorganic Se to

animal feed is most likely an economic one (Pehrson, 1993; Mahan, 2001). According to Mahan (2001), sodium selenite and sodium selenate are equally effective, but sodium selenite is used more often because it is less expensive than sodium selenate. Sodium selenite is water soluble and could be toxic to humans if inhaled or if it comes in contact with skin. Other inorganic forms, such as calcium selenite and barium selenite are less water soluble and therefore less toxic to handlers, but are not approved by the FDA for use in swine (Mahan, 2001).

Research is now showing that the use of inorganic sources of Se have some significant limitations that include potential toxicity, interactions with other minerals, poor retention, low efficiency of transfer to milk and meat, and poor ability to maintain Se reserves in the body (Surai, 2000). Spallholz (1997) adds that the prooxidant status of sodium selenite is another major disadvantage to feeding this form of Se. Unlike selenite, selenate and SeMet have not been shown to generate superoxide radicals in vitro. To avoid some of the potential problems with the use of sodium selenite, manufacturers began producing organic Se supplements that primarily consist of SeMet (Spallholtz, 1997).

Selenomethionine can also be fed in supplemental form through feeding Se-enriched yeasts, which are the product of aerobic fermentation of *Saccharomyces cerevisiae* yeast grown in a Se-enriched medium. According to Rayman (2004), different companies use different strains of *S. cerevisae* and may describe them as either bakers or brewers yeast. Beet or cane molasses media is typically supplemented with vitamins, nutritional salts, including sodium selenite, and other growth factors to maximize yeast production. The yeast cream that is produced is then pasteurized to kill the yeast, and then typically spray dried to dry the product. The Se becomes bound to the yeast during the fermentation process. According to Rayman (2004), the amount of Se bound to the yeast should be at least 90%, but this can fluctuate on a

batch to batch basis, as well as vary on a manufacturer to manufacturer basis. Reputable manufacturers carry out quality control tests that assess the purity of the yeast strain, the percentage of complexed Se, and the percent of SeMet in the final product.

In a survey of five Se-yeast products, Rayman (2004) found varying amounts of SeMet, ranging from 60 or 61% (Selenomax; Cypress Systems, Fresno, CA) to 84% (SelenoExcel; Cypress Systems, Fresno, CA). One of the most well-known Se-yeast products used as an organic Se supplement in animal diets is Sel-Plex (Alltech Inc., Nicholasville, KY), which is made up specifically of *S. cerevisa* strain CNCM I-3060. Sel-Plex was approved by both the FDA (FDA, 2003) and the European Union (EU, 2006) as feed additives in livestock diets. According to a personal communication (Ronan Power, Alltech Inc.) referenced by Rayman (2004), Sel-Plex contains 83% SeMet, 0.3% selenite and 5% SeCys, which adds up to the sum of identifiable Se-containing chemical species of 88.3%. That leaves 11.7% of the Se-species within Sel-Plex unidentified. It is important to note that comparison between products is very difficult because of varying extraction techniques and analytical methods used in different laboratories. Another Se-yeast product of unknown composition called SelenoSource (Diamond V Mills, Cedar Rapids, IA) is also currently available within the U.S.

Se Absorption and Metabolism. As with many trace minerals, the absorption and metabolism of Se is dependent on the amount and chemical form of Se fed, other dietary components and species of animal. Organic Se, found in grains, forages, and other feed ingredients, consists primarily of SeMet and is metabolized in the same way as methionine with active transport in the gut. In this manner, SeMet and methionine compete with each other for the same absorption site. Groce et al. (1973) reported that the seleno amino acids from grain-based organic Se are absorbed at a slower rate than inorganic sources. Selenium from plant

sources generally have a greater absorption rate (> 60%) compared to Se from animal sources (< 25%). This is dependent on the source of animal Se being fed, however. According to Mathias et al. (1967), the absorption of Se from bovine milk is similar to that of selenite, while the absorption of Se from meat and bone meal and poultry byproducts is less than 20%.

The absorption of selenite is similar to other minerals that are passively transported across the gut. The absorption of selenate, on the other hand, is sodium dependent and relies on active transport mechanisms along the brush border membrane of the ileum. Arduser et al. (1986) determined that the uptake of selenate and selenite occurred at a faster rate in the rat small intestine compared to the sheep small intestine. They also found that mucosal selenate uptake across the intestinal brush border was Na^+-dependent in all portions of the rat small intestine and the sheep jejunum and ileum. Uptake of selenite was Na^+- independent throughout the rat small intestine and the sheep duodenum and ileum, whereas uptake of selenite in the sheep midjejunum was shown to be Na^+-dependent. The authors suggested that the Na^+-independent mucosal uptake of selenite or selenate probably represents diffusion. The Na+-dependent transport mechanism has an affinity for other anions such as chromate, thiosulfate, sulfate, and molybdate, which were shown to affect selenate and selenite transport across the intestinal brush border. Sulfate and chromate inhibited selenate transport in the midjejunum of sheep to a greater extent than that of molybdate. In the rat ileum, chromate and sulfate inhibited selenate transport to a greater extent than that of sulfate which subsequently inhibited transport to a greater extent than that of molybdate. Thiosulfate also inhibited mucosal selenite uptake in the sheep midjejunum. Preincubation of rat ileum with glutathione enhanced uptake of selenite, whereas selenate uptake was unaffected. The mechanism behind glutathione enhancing selenite uptake is probably due to an increase in intracellular selenite metabolism.

Balance studies based on the disappearance of Se from the gastrointestinal tract have shown efficient absorption of both inorganic and organic sources of Se. Thomson and Stewart (1972) determined that rats absorbed 91 to 93% of dietary selenite and 95 to 97% of dietary selenomethionine. In a subsequent experiment, Thomson et al. (1975) compared the absorption of radioactively-labeled SeMet and SeCys. In these experiments, 86% and 81% of the doses of SeMet and SeCys, respectively, were absorbed within the gastrointestinal tract of rats. When examining total excretion of ^{75}Se after 1 wk, 29.6% was excreted in the urine and 70.4% was excreted through the feces. Of the Se excreted in the feces, 49.1% was unabsorbed fecal ^{75}Se and 21.2% was endogenous fecal Se. When [^{75}Se]SeMet was administered for 7-d, 18.4% of ^{75}Se was excreted in the urine and 81.6% was excreted through the feces, with 50% representing unabsorbed fecal ^{75}Se and 31.6% representing endogenous fecal Se. Within the first wk of administration, it was calculated that 61.5 to 62.1% of the [^{75}Se]SeCys dose was retained, compared to 72.8 to 77.0% retention when [^{75}Se]SeMet was administered ($P < 0.001$). After the first week, [^{75}Se]SeCys and [^{75}Se]SeMet were metabolized similarly, suggesting that they are incorporated into the same metabolic pool of Se.

Once dietary selenate is absorbed it is reduced to selenite, which is then converted to selenide (H_2Se). Selenomethionine, which can come from body stores of methionine proteins or from dietary sources, is converted to selenocysteine, which is subsequently converted to selenide. Selenide is the biologically active form of selenium in the body. However, selenide itself is not readily absorbed. Selenide has one of two fates: excretion or selenoprotein synthesis. If selenide is to be excreted, a series of sequential methylation reactions converts selenide to methyl selenol (CH_3SeH), then dimethyl selenol ($[CH_3]_2Se$), and then trimethylselenonium

($[CH_3]_3Se^+$), which is excreted in the urine. Dimethyl selenol can also be excreted via the lungs. Selenomethionine can also be directly excreted through the feces (Surai, 2006).

Selenium balance has been shown to be affected by Se source. Ku et al. (1973) found that tissue Se concentrations resulting from supplementing 0.4 ppm sodium selenite to a Se-deficient diet (0.04 ppm Se) were lower than when a diet naturally high in Se (0.44 ppm Se) was fed to pigs. Mahan and Parrett (1996) fed crossbred growing-finishing barrows a basal diet (0.039 to 0.058 ppm Se) with one of seven treatments: no supplemental Se, organic Se (0.1, 0.3, and 0.5 ppm Se), or inorganic Se (0.1, 0.3, and 0.5 ppm Se). Throughout a 7-d metabolism trial during which feces and urine were collected, Se retention increased as dietary Se levels increased, particularly when the organic Se was fed, resulting in a dietary source by Se level interaction. As dietary Se levels increased, urinary Se increased to a greater extent when pigs were fed sodium selenite, whereas fecal Se increased to a greater extent when the organic Se source was fed. When total Se excretion was compared between Se sources and dietary levels, 16 to 19% less Se was excreted when the organic Se was fed. The apparent absorption of Se was higher when the sodium selenite was fed but tissue Se retention was greater when the organic Se was fed. These results agree with those of Groce et al. (1973), Parsons et al. (1985) and Tian et al. (2006).

Kim and Mahan (2001c) investigated the impact of feeding barrows a corn and soybean meal-based basal diet supplemented with one of two sources of Se (selenized yeast or sodium selenite) at a wide range of Se levels (0.3, 1.0, 3.0, 5.0, 7.0, and 10.0 ppm Se). The results from the Se treatments were not compared to a control unsupplemented diet. Urinary Se increased as dietary Se level increased, but increased more and at a higher rate when sodium selenite was fed, resulting in a form of Se supplementation by level interaction. Urinary Se excretion was

approximately 25% higher when pigs were fed sodium selenite. When considering these values as percent of intake, approximately 50% of the consumed inorganic Se was excreted via the urine, whereas 20% of the consumed organic Se was excreted through urinary routes. Fecal Se increased linearly as the dietary Se level increased, but fecal Se increased at a faster rate when selenized yeast was fed, resulting in an interaction between Se form and supplementation level. When total Se excretion (urinary and fecal excretion) was considered, approximately 68% of the consumed Se from inorganic Se was excreted, whereas 64% was excreted when the organic Se was fed. Se retention increased linearly as dietary Se increased with either Se source. The apparent digestibility of Se increased with Se level when pigs were fed inorganic Se, but not when the organic Se source was fed.

Groce et al. (1973) also reported that storage conditions influence the tissue retention and excretion of sodium selenite. In this experiment, nine pigs were assigned to one of three dietary treatments: I) a basal corn-soybean meal diet (0.04 ppm Se) supplemented with 0.2 ppm sodium selenite mixed in a glucose premix that had been stored for 9 mo in a brown glass jar at ambient temperatures, II) the basal diet supplemented with 0.2 ppm sodium selenite mixed in a freshly prepared glucose premix, and III) the basal diet supplemented with 0.2 ppm sodium selenite and 22 IU vitamin E/kg diet mixed in a freshly-prepared glucose premix. Urinary Se excretion increased ($P < 0.05$) and Se retention decreased ($P < 0.01$) for the pigs fed the stored sodium selenite-glucose premix compared to the freshly made premix. However, when Se retention was expressed as a percent of intake, there were no differences in Se retention between the treatments. One important note to make about this study is that only three pigs were assigned to each treatment, and all of the pigs used came from the same litter.

Selenoproteins. Selenium participates in a variety of physiological functions as an integral part of selenoproteins. The Se-dependent SeCys moiety, often included in the active sites of these enzymes, is essential for the catalytic function of selenoproteins. As a result of its importance in selenoprotein function, SeCys has been referred to as the 21st amino acid. In humans, 25 selenoproteins have been identified and include: five unique glutathione peroxidases, three thioredoxin reductases, three iodothyronine deiodinases, a single selenophosphate synthetase enzyme, and 13 additional selenoproteins. Nine additional selenoproteins have been identified in zebrafish, *Drosophilia melanogaster*, chickens, fish, and rats. Another 19 selenoproteins have been identified in prokaryotes (Behne and Kyriakopoulos, 2001; Surai, 2006; Lu and Holmgren, 2009).

Glutathione peroxidases are known to be major components of antioxidant defenses. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, protecting cells from oxidative damage. The primary GPx are as follows: cytosolic or classical GPx (GPx1), gastrointestinal GPx (GPx2), plasma GPx (GPx3), phospholipid hydroperoxide GPx (GPx4), and sperm nuclei glutathione peroxidase (snGPx). There are three iodothyronine deiodinases that catalyze the activation and inactivation of the thyroid hormones regulating various metabolic activities: type 1 deiodinase (D1), type 2 deiodinase (D2), and type 3 deiodinase (D3). These deiodinases differ in regard to tissue distribution and the role of deiodination of thyroxine and its metabolites. There are three thioredoxin reductases that are the only enzymes able to reduce oxidized thioredoxin. These include thioredoxin 1 reductase, a cytosolic enzyme, thioredoxin reductase 2, a mitochondrial enzyme, and thioredoxin reductase 3, a enzyme preferentially expressed in the testes. Selenophosphate synthetase 2 is an enzyme that catalyzes the reaction of selenide with adenosine

monophosphate, producing selenophosphate, which acts as the donor for the biosynthesis of selenocysteine. Additional selenoproteins, which include selenoprotein P, W, the 15-kDa selenoprotein, and the 18-kDa selenoprotein have unknown functions (Behne and Kyriakopoulos, 2001; Surai, 2006; Lu and Holmgren, 2009).

One problem with classification of these selenoproteins is that references used the GPx nomenclature differently. In the 1980's and 1990's when there was only one known GPx, many scientists investigated the impact of Se on what is now believed to be GPx1. Some references (Surai, 2000, 2006) list GPx2 as the gastrointestinal GPx and GPx3 as the plasma GPx, however other references (Rao et al., 2001) list these two GPx oppositely, with GPx2 as the plasma GPx and it would assume GPx3 as the gastrointestinal GPx. For the purposes of this review, GPx2 will be referred to as the gastrointestinal GPx, and GPx3 as the plasma GPx.

Selenium can be included into selenoproteins through a number of different routes. After ingestion of selenite, selenate, or SeCys, nearly all of the Se is transported via an intermediary pool into specific SeCys-containing selenoproteins, which are responsible for its biological effects. This intermediary Se pool can also be included into specific Se-binding proteins or included into Se-containing proteins via nonspecific binding. Unlike selenate and SeCys, selenite has the ability to bypass the intermediary Se pool and be directly incorporated into specific Se-binding proteins. When SeMet is available in the diet, SeMet is nonspecifically incorporated into a variety of methionine proteins in place of methionine. This could be a way of preserving Se for future use in the body. The selenium in SeMet becomes available for selenoproteins when those selenoproteins are catabolized to selenide (H_2Se) within proteasomes. Another portion of dietary SeMet is metabolized in the same way as other Se compounds via the intermediary Se pool. There are also selenoproteins in which selenium has been detected, but for

which no information on binding is currently available (Behne and Kyriakopoulos, 2001; Schrauzer, 2003; Surai, 2006).

Selenoprotein synthesis. The unusual feature of selenoprotein synthesis is that SeCys insertion into selenoproteins is specified by a stop UGA codon in the open reading frame of mRNA. Selenocysteine differs from other amino acids in that it is synthesized on its specialized tRNA, $tRNA^{[Ser]Sec}$. The first step in selenoprotein synthesis is the charging of serine (Ser) on the specialized $tRNA^{Sec}$ to produce seryl-$tRNA^{Sec}$. Selenocysteine synthase catalyzes the conversion of seryl-$tRNA^{Sec}$ to selenocysteyl-$tRNA^{Sec}$ (Sec-$tRNA^{Sec}$). Selenocysteine is then cotranslationally incorporated into selenoprotein active centers (Schrauzer, 2003; Surai, 2006).

During selenoprotein synthesis, selenide is converted to selenophosphate, which is subsequently used for the synthesis of SeCys on seryl-$tRNA^{Sec}$. Selenocysteine is then inserted into a growing polypeptide chain when the UGA codon of the specific tRNA is read (Schrauzer, 2003).

In addition to Sec-$tRNA^{Sec}$, which carries the anticodon for UGA, it has been theorized that eukaryotic selenoprotein biosynthesis requires a complex of the selenocysteine insertion sequence (SECIS), SECIS-binding protein 2 (SBP2), and a selenocysteine-specific elongation factor (eEFSec). This model requires a looping out of the mRNA such that the loaded SECIS element in the 3' untranslated region is positioned at the upstream UGA codon. It appears that SBP2 recruits the elongation factor to the selenoprotein mRNA. Copeland (2003) suggested that SBP2/SECIS complex interacts with eEFSec/Sec-tRNASec complex to deliver SeCys to SECIS containing mRNA. In the absence of a functional SECIS element, or under conditions of limiting selenoprotein biosynthesis or incorporation, the UGA codons in these messages specify termination. Research has also shown that SBP2 preferentially stimulates incorporation directed

by selenoprotein P and GPx4 SECIS elements over those of other selenoproteins (Low et al., 2000).

III. Effect of selenium supplementation on growth performance

Effect of selenium supplementation on growth performance of barrows and gilts.

Numerous studies have shown that Se supplementation had no impact on barrow or gilt growth performance (Wastell et al; 1972; Wilkinson et al., 1977b; Meyer et al., 1981; Adkins and Ewan, 1984; Goehring et al., 1984a; Mahan, 1985; Suomi and Alaviuhkola, 1992; Mahan and Parrett, 1996; Ortman and Pehrson, 1998; Mahan et al., 1999; Wolter et al., 1999; Kim and Mahan, 2001c; Mahan and Peters, 2004; Mateo et al., 2007).

Mahan and Peters (2004) failed to detect differences in ADG, ADFI, or G/F of gilts fed a basal diet (0.065 to 0.075 ppm Se) with no supplemental Se, 0.15 ppm or 0.3 ppm organic Se, 0.15 ppm or 0.3 ppm inorganic Se, or a combination of 0.15 ppm inorganic and 0.15 ppm organic Se during the growing-finishing period (27 to 115 kg BW). Similarly, Mahan and Parrett (1996) and Mahan et al. (1999) found no differences in ADG, ADFI or G/F of crossbred pigs fed a basal diet with no supplemental Se (0.04 to 0.08 ppm Se), organic Se (0.05, 0.1, 0.2, 0.3, and 0.5 ppm Se), or inorganic Se (0.05, 0.1, 0.2, 0.3, and 0.5 ppm Se). Mateo et al. (2007) compared growth performance in pigs fed a basal corn-soybean meal diet with higher endogenous Se (0.181 ppm Se) to pigs supplemented with 0.1, 0.2, or 0.3 ppm organic Se, or 0.3 ppm inorganic Se. There was no effect of treatment on ADG, ADFI or G/F. Although no control unsupplemented diet was fed, Wolter et al. (1999) also failed to find differences in growth performance of gilts supplemented with 0.3 ppm sodium selenite or 0.3 ppm Sel-Plex. Increasing Se concentrations to 5 ppm Se did not affect the growth performance of gilts or barrows (Goehring et al., 1984b; Mahan and Magee, 1991; Kim and Mahan, 2001a).

Tian et al. (2006) conducted an experiment using a 3 x 2 factorial arrangement of treatments, with different Se sources as one factor (sodium selenite, Se-enriched yeast, and a second unknown organic Se product) and dietary inclusion level (0.1 or 0.3 ppm Se) as the second factor. The experiment also included a non-Se-fortified basal diet that served as a negative control. In the growing phase (0 to 6 wk), ADFI increased ($P < 0.05$) when pigs were fed organic Se compared to pigs fed the control or inorganic Se diet. Pigs fed inorganic Se had greater ($P < 0.05$) ADFI compared to pigs fed organic Se during the late finishing phase (7 to 12 wk). When ADFI was considered throughout the whole experimental period there were no differences due to Se source. Growth performance variables including BW, ADG, and G/F were not affected by Se source or dietary inclusion levels.

Increasing the level of Se from 0.3 to as much as 20 ppm Se, either in the form of sodium selenite or calcium selenite, led to linear decreases in ADG, ADFI, and G/F ratios (Goehring et al., 1984b; Mahan and Magee, 1991). When the dietary levels were increased to 10, 15, or 20 ppm, ADG declined more rapidly when the inorganic Se source was fed compared to when the organic Se source was fed, resulting in a dietary Se source by Se inclusion level interaction (Kim and Mahan, 2001a).

Although there was no difference in gilt and barrow growth performance with Se supplementation up to 5.0 ppm, this may not necessarily be the case for boars. Of the sexes, boars have been shown to be leaner than gilts, which in turn are leaner than barrows. The magnitude of difference in leanness between the sexes increases as the animals increase in weight (Nold et al., 1997, 1999).

Effect of selenium supplementation on boar growth performance. Growth rates, feed intakes, and feed conversion efficiencies were similar for control boars and boars provided

supplemental inorganic Se in the diet (0.5 ppm; Kolodziej and Jacyno, 2005; Marin-Guzman et al., 1997) or via s.c. injections (0.33 ppm as sodium selenite) at 14-d intervals (Segerson et al., 1981). In contrast to these reports, Henson et al. (1983) reported that boars fed diets supplemented with Se exhibited some signs of retarded growth. In that study, boars were fed a basal corn and soybean meal diet (0.05 ppm Se) or the basal diet supplemented with a Se premix at concentrations of either 0.10 or 0.25 ppm. While there was no effect of treatment alone, treatment by age interactions existed for body weight. Body weights were greatest for boars consuming the basal diet alone, intermediate for the boars supplemented with 0.1 ppm Se, and least for boars fed the diet supplemented with 0.25 ppm Se. Jacyno et al. (2002) reported that boars fed a wheat, barley, and triticale-based basal diet supplemented with 0.2 ppm sodium selenite and 30 IU vitamin E gained weight faster and more efficiently than boars fed the basal diet supplemented with 0.2 ppm organic Se and 60 IU vitamin E. There was an interaction between season and dietary treatment, with the boars consuming the inorganic source of Se gaining more weight on a more efficient basis during January to April compared with June to September. These data, however, must be interpreted with caution because Se source was confounded with level of vitamin E.

IV. Effect of dietary selenium supplementation on carcass characteristics

Effect of selenium supplementation on carcass characteristics in barrows and gilts. In general, Se level and Se source has failed to affect carcass characteristics of barrows and gilts (Ortman and Pehrson, 1998; Mahan et al., 1999; Mateo et al., 2007).

In contrast to these reports, Wolter et al. (1999) supplemented gilt diets with either 0.3 ppm sodium selenite or Sel-Plex. No differences ($P \geq 0.22$) were found for cold carcass weight, dressing percentage, carcass length, 10th rib backfat thickness, loin pH, Hunter L* score, drip

loss, cooking loss, or water holding capacity. However, gilts fed the organic Se source had greater ($P = 0.04$) loin eye areas (42.78 cm^2) compared to gilts fed sodium selenite (39.88 cm^2). Gilts fed sodium selenite also had increased ($P = 0.03$) last lumbar backfat depths (14.1 mm) compared to those fed Sel-Plex (11.8 mm). The protein content of a loin chop tended to be greater ($P = 0.08$) for gilts fed Sel-Plex (22.84 ± 0.38 %) compared to those fed sodium selenite (22.24 ± 0.38%). There was no difference ($P > 0.15$) due to dietary treatment in the water or fat content of loin chops. It is important to note that these investigators did not compare the Se-supplemented diets to a control, unsupplemented diet.

Mahan et al. (1999) reported that drip loss, which represents the water-holding capacity of loin muscle, 24 to 72 h after slaughter tended to be higher when sodium selenite was fed, compared to when organic Se was fed. The drip loss from the loin of sodium selenite-fed pigs was similar to that of the pigs consuming the basal diet containing 0.06 ppm Se. Retaining the pork loin in the cold room or freezer from 72 to 120-h after slaughter did not result in differences in drip loss between the dietary treatments. For the overall 24- to 120-h period, pigs fed the inorganic Se tended to have a higher water loss from the loin compared to carcasses from pigs fed organic Se. Similarly, Mateo et al. (2007) found that carcasses of organic Se-fed animals had reduced 48-h drip loss percentages compared to those animals consuming the nonfortified diets. Furthermore, percent drip loss was reduced linearly as added dietary Se concentration increased. Drip loss of loins was not different between unsupplemented pigs fed the control diet or when pigs were fed 0.3 ppm sodium selenite. Zhan et al. (2006) reported similar findings and Bobcek et al. (2004) reported a tendency for organic Se to reduce loin drip loss. The discrepancy between these two reports (Mahan et al., 1999; Mateo et al., 2007) could be associated with

starting Se level in the diet, as the basal diet from Mateo et al. (2007) contained 0.181 ppm Se compared to the basal diet of Mahan et al. (1999) which contained 0.06 ppm endogenous Se.

Pigs fed sodium selenite had a lighter, or more pale colored muscle, that increased linearly as dietary Se level increased. This phenomenon was not seen with the loins from carcasses of pigs fed the organic Se (Mahan et al., 1999). Zhan et al. (2006) reported that loins stored in a 25˚C room for 45 min had increased Hunter a (redness) values in barrows fed either 0.3 ppm selenomethionine or sodium selenite compared to control animals consuming a basal diet (0.045 ppm Se). After 8-h of storage, loins from barrows fed selenomethionine had greater Hunter a values compared to control animals. After 16-h storage, loins from selenomethionine-supplemented barrows had higher Hunter a* values compared to control or selenite-supplemented barrows.

Nuernberg et al. (2002) reported that gilts fed a barley, wheat, and soybean meal-based diet supplemented with 0.3 ppm Se (form unreported had decreased linolenic (C18:3) fatty acid content of loin muscle microsomes. Stearic (C18:0), oleic (C18:1 *cis*-9), linoleic (C18:2), arachadonic (C20:4), total omega 3 fatty acid, and total polyunsaturated fatty acid content of the muscle microsomes were not changed by Se supplementation. Selenium-supplemented gilts exhibited increased stearic, linoleic, and polyunsaturated fatty acid content in loin muscle mitochondria. Oleic, linolenic, arachadonic acid, and total omega-3 content of muscle mitochondria were not affected by Se supplementation. Loin muscle microsome membrane fluidity, measured by the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene, was also unaffected by Se-supplementation. Malondialdehyde-induced lipid peroxidation of loin muscle samples from Se-supplemented gilts was not different from control or vitamin E (200 mg/kg diet) supplemented gilts. However, Se-supplemented and control gilts had greater accumulation of

thiobarbituric acid reactive substances (TBARS) after incubation of loin muscle homogenates in iron sulfate and ascorbate for up to 3-h compared to vitamin E-supplemented gilts.

Bugel et al. (2004) reported that the apparent absorption of Se was 94% on average when humans consumed pork from gilts fed a barley, wheat, and soybean-based basal diet (0.26 ppm Se), or the basal diet supplemented with 6% canola oil, or the basal diet supplemented with 6% canola oil and 200 mg vitamin E. However, none of these diets were from Se-supplemented animals.

Effect on boar carcass characteristics. Much less is known about the effect Se may have on carcass characteristics in boars. Jacyno et al. (2002) reported that boar meatiness, determined ultrasonically at 180 days of age, was not different between boars receiving 0.2 ppm selenite + 30 IU vitamin E and boars receiving 0.2 ppm organic Se and 60 IU vitamin E.

Tissue selenium concentrations. The Se status of animals is greatly influenced by the Se content of plant materials and supplementary forms of Se consumed. Tissue data indicates that tissue Se levels are generally highest when organic Se is fed. While some SeMet is converted into SeCys for incorporation into selenoproteins (glutathione peroxidase, thioredoxin reductase, etc.), much of the Se consumed is directly deposited into tissue. When sodium selenite is fed a larger proportion of the Se is incorporated into selenoproteins, leaving Se tissue deposition lower (Mahan and Parrett, 1996).

Prior to the FDA regulations allowing the inclusion of Se in swine diets, Ku et al. (1972) reported a large variation in longissimus muscle Se content from pigs depending on the state they originated from. Dietary Se levels were greatest from South Dakota (0.493 ppm Se), North Dakota (0.412 ppm), and Nebraska (0.330 ppm) compared to diets from Idaho (0.086 ppm), Indiana (0.052 ppm), Michigan (0.040 ppm), Illinois and New York (0.036 ppm), and Virginia

(0.027 ppm). Pigs consuming grains with high indigenous Se contents had greater longissimus muscle Se content compared to pigs consuming low Se diets. To further investigate this phenomenon, Mahan et al. (2005) surveyed diets and tissue Se content in 19 states in north, central, and southern regions of the U.S. States in the west-central region of the U.S. and west of the Mississippi River had higher dietary Se and tissue Se concentrations compared to states in the eastern section of the Corn Belt region, east of the Mississippi River, and along the East Coast of the U.S. Dietary Se content was highly correlated with loin ($r = 0.84$), heart ($r = 0.84$), liver ($r = 0.83$), and hair Se ($r = 0.90$) concentrations.

Segerson et al. (1981) reported that injecting boars with 0.33 ppm sodium selenite increased the Se concentrations in serum, kidney, liver, heart, skeletal muscle, testis, epididymis, prostate, seminal vesicles, bulbourethral glands, whole semen, spermatozoa, and seminal plasma compared to boars receiving a saline injection and a basal cornstarch-torula yeast diet (0.025 ppm Se). A similar pattern of tissue Se distribution in kidney, liver, and muscle was shown after a single intramuscular injection of sodium selenite (0.6 mg/kg BW; Lindberg and Tanhuanpaa, 1965) or when feeding diets supplemented with sodium or calcium selenite at 0.3 ppm (Mahan and Magee, 1991). However, including dietary sodium or calcium selenite at 5.0 or 15.0 ppm increased liver Se levels above those seen in the kidney (Mahan and Magee, 1991).

Mahan and Parrett (1996) and Mahan et al. (1999) reported that kidney, liver, and loin Se concentrations were highest in growing-finishing pigs fed 0.3 ppm organic Se, intermediate from pigs fed 0.3 ppm sodium Se, and lowest from pigs fed the unsupplemented basal diet that contained 0.06 ppm Se. Regardless of the source and level, kidney Se levels were greatest, followed by liver levels, and loin Se levels were the lowest.

Kim and Mahan (2001b) investigated the impact of feeding different levels of Se, two Se sources, and hair color on indicators of Se status in growing-finishing pigs. The first experiment was conducted in two replicates and utilized a 2 x 6 x 2 factorial arrangement of treatments in a split-plot design. Sodium selenite and Se-enriched yeast added at 0.3, 1, 3, 5, 7, and 10 ppm Se served as the main plot and pig hair color as the subplot. Plasma Se increased as dietary Se level increased, when organic Se was provided, and was higher when pigs were white-haired compared to dark-haired (red or black) pigs. A time by hair color by dietary Se level interaction occurred, in which hair Se concentration was higher in dark- than in white-colored pigs and increased as dietary Se level increased and as the experiment progressed. Correlation coefficients between dietary Se source and hair Se content of growing finishing pigs were greater in white-haired pigs (r = 0.95) and dark-haired pigs (r = 0.97) fed organic Se compared to white-haired pigs (r = 0.80) and dark-haired pigs (r = 0.90) fed inorganic Se. A second experiment utilized a 3 by 6 factorial arrangement of treatments in a split-plot design with three 9-month old gilts from each of the Yorkshire, Duroc, and Hampshire breeds to determine whether hair Se concentration varied between body location and breed. Hair Se was higher in red- and white-haired gilts compared to black-haired gilts. Hair collected in the lower body region (limbs and belly) had higher Se levels compared to hair collected along the upper body region (shoulder, topline, and rump). The difference due to hair location could be partially explained by a greater possibility of contamination by urine and/or feces along the lower body region.

V. Effect of dietary selenium supplementation on reproductive performance

Effect of selenium supplementation on sow and gilt reproductive performance. Wahlstrom and Olson (1959) investigated the impact of high levels of dietary Se (10 ppm) on gilts and the value of arsanilic acid (0.1%) to prevent Se poisoning. Selenium supplementation

lowered the conception rate and increased the number of services required per conception, but it was not mentioned whether this difference was statistically significant. Sows fed 10 ppm Se farrowed a numerically higher percentage of dead pigs. Surviving pigs were smaller and weaker at birth in the first litter, but not the second litter. Fewer pigs were weaned and the 56-d weights of these pigs were also reduced in both the first and second litters. Numerically the addition of arsanilic acid in the Se ration improved the number of pigs alive at farrowing, but did not improve birth weights in the sow's first litter. Arsanilic acid also increased the weaning weight of pigs in the sow's first litter.

Mahan and Kim (1996) fed two sources of Se (selenite and Se-enriched yeast) at two levels (0.1 and 0.3 ppm) to 43 first-parity gilts to evaluate the effect of Se on gilt reproductive performance. Treatment diets, beginning 60 d prior to breeding, had no effect on gilt reproductive performance. Gilt weights and weight changes that occurred during gestation and lactation were not influenced by Se treatments. The number of pigs born (total, live, and stillborn) and individual pig birth weights were not affected by Se source or level. Litter birth weights were numerically higher with gilts fed 0.3 ppm of either Se source, but the difference was not statistically significant. These results concur with those reported by Wilkinson et al. (1977a).

A study conducted by Mahan et al. (1975) found no significant differences in first parity pig weights and litter sizes when sows were fed a corn-soybean meal-based basal diet or the basal diet supplemented with 0.1 ppm sodium selenite. Also in parity one, only three sows farrowed out of nine bred and litter sizes decreased when sows were fed a semi-purified diet devoid of supplemental Se or vitamin E compared to those fed the corn-soybean meal diets. While pig birth weight and weight gain was not affected, total number of pigs born was reduced

in sows fed the unsupplemented corn-soybean meal diet compared to Se-supplemented sows in their second parity. Of the sows fed the semi-purified diet, none of the sows farrowed out of six sows that were bred, with three dying of Se-vitamin E deficiency syndrome during gestation, one each at 10 d, 88 d, and 105 d post-breeding.

Mahan and Peters (2004) found that the number of total and live pigs was numerically smaller when the non-fortified basal diet (0.055 to 0.085 ppm Se) was fed compared to sows fed organic Se (0.15 or 0.3 ppm Se), inorganic Se (0.15 or 0.3 ppm Se), or a combination Se (0.15 ppm inorganic Se + 0.15 ppm organic Se), but there was no difference due to Se treatment or Se level fed. Sows fed inorganic Se alone had greater numbers of stillborn pigs per litter. Litter birth weight, but not individual pig birth weight, was reduced with sows fed the basal diet compared to the fortified diets, but this was most likely due to the smaller litter sizes seen with the unsupplemented sows. The percentage of neonatal pigs exhibiting the splay-legged condition was numerically greater with sows fed the basal or inorganic Se diets, but this response was not significant. Pig weight gain and litter ADG were not affected by treatment. Over four parities, sow BW and weight changes during various measurement periods were not affected by dietary Se source or Se level. Sow feed intake and backfat thickness differed at the various measurement periods, but there was no consistent response to dietary Se. The number of days from weaning to rebreeding was not affected by treatment.

Mahan and Peters (2004) also found that as the sow parity increases, milk Se concentration decreases when the diet is supplemented with sodium selenite. There are three reasons for this effect. First, tissue reserves of Se are depleted with increasing parity in sows. Secondly, tissue reserves are not readily mobilized. Lastly, mammary tissue of older females may not incorporate or transfer dietary inorganic Se or tissue Se reserves into milk as effectively

as young sows. Their results also showed that Sel-Plex was more effectively transferred to colostrum, milk, and sow hair, compared to sodium selenite. Higher milk Se concentrations have been shown to improve the Se status in the nursery pig at weaning (Mahan and Kim, 1996). Higher Se status may help reduce the occurrence and severity of post-weaning mortality, a problem commonly found in commercial nurseries.

Effect of selenium supplementation on reproductive performance in males. Bartle et al. (1980) did not find a difference in postcollection or post-thaw sperm characteristics of mature Holstein bulls injected with 0.055 ppm Se (as sodium selenite) at the start of the experiment followed by injections containing 0.11, 0.22, and 0.44 ppm Se at 10, 16, and 22 wk after the first injection, respectively. However, only three bulls were injected with Se and two bulls received the sham polysorbate injection. Segerson and Johnson (1981) injected 12 yearling Angus bulls with 50 mg of sodium selenite and 680 IU vitamin E initially and then repeated injections of 30 mg Se and 408 IU vitamin E every 21 d for approximately 150 d; a second group of 12 yearling bulls was administered vehicle. After the 150 d, the bulls were electroejaculated, at which time semen was processed and frozen for subsequent examination, and the bulls were harvested for tissue collection. Semen characteristics of the fresh ejaculates prior to freezing were not recorded. Post-thaw semen viability and gross morphology characteristics were not different among treatments. There was also no difference in testis or epididymal weight or sperm concentration within the testis or epididymis.

Kaur and Bansal (2004a) fed mice a basal yeast-based diet (0.02 ppm Se) and supplemented the basal diet with either 0.2 or 1.0 ppm Se (sodium selenite). They reported significant reductions in sperm motility (58.0% vs. 90.6% and 85.5% for control, 0.2 ppm Se, and 1.0 ppm Se, respectively) and total sperm numbers in their ejaculates (0.73 x 10^6/mL vs.

2.05 x 10^6/mL and 1.73 x 10^6/mL, respectively). Interestingly, the researchers reported seeing increased DNA fragmentation in genomic DNA extracted from testicular tissue from mice fed the unsupplemented basal diet compared to mice supplemented with 0.2 ppm Se. Mice supplemented with 1.0 ppm Se also exhibited increased DNA fragmentation compared to mice supplemented with 0.2 ppm Se, but the amount of DNA fragmentation was less than that which was seen with the unsupplemented mice.

Watanabe and Endo (1991) fed mice either a basal torula yeast-based diet (containing < 0.01 ppm Se) or the basal diet supplemented with 0.5 ppm Se (sodium selenite). Results from cytogenetic tests of metaphase-1 spermatocytes with abnormal chromosomes did not differ between the Se-deficient and the Se-supplemented mice, and were comparable to data previously reported in mice. Selenium deficient mice had larger numbers (19.7%) and a larger variation of abnormal sperm (ranging from 6.8% to 49.6%) compared to supplemented mice (7.7%), which also had a smaller variation (4.0% to 15%). The most common morphological abnormality were those in the sperm head, including abnormal head, giant, or dwarf heads, and was greater for Se-deficient mice (13.6%) compared to Se-supplemented mice (4.1%). Abnormalities of other regions such as the neck, midpiece, or tail tended to be greater for Se-deficient mice compared to Se-supplemented mice, and were mainly attributed to bending, torsion, or coiling. The incidences of these abnormalities were relatively low, however, even for the Se-deficient group.

Kaur and Bansal (2005) found significant decreases in the number of pachytene spermatocytes and young and mature spermatids in mice fed a basal yeast-based diet with no additional Se compared to mice fed the basal diet and 0.2 ppm sodium selenite. Dietary treatments did not affect populations of A spermatogonia, preleptotene spermatocytes, predecessors of the pachytene spermatocytes, or Sertoli cells. Several groups have demonstrated

that pachytene spermatocytes and round spermatids, in contrast to spermatogonia, have decreased capabilities or are even unable to repair DNA damage, suggesting that these cells are more vulnerable to oxidative stress than are other germ cells (Oldereid et al., 1998). Kaur and Bansal (2005) also showed that the unsupplemented mice had lower numbers of spermatozoa and exhibited 50% reductions in fertility and reductions in litter sizes compared to mice supplemented with 0.2 ppm Se. There was no additional benefit in germ cell populations, total spermatozoa, or fertility when mice were supplemented with 1.0 ppm sodium selenite.

Kaur and Kaur (2000) showed that adult male albino rats fed excess dietary Se (6 or 8 ppm as sodium selenite) had reduced BW, reduced testicular and cauda epididymal weight, and increased number of morphologically abnormal spermatozoa. There was a considerable time- and dose-effect seen in the decreases in seminiferous tubule diameter, seminiferous epithelial heights, lumen diameters, and reductions in numbers of spermatogenic cells seen in rats fed 6 or 8 ppm Se. Cauda epididymal tubules also exhibited reductions in diameter, epithelial height, and lumen diameter. While offspring birth weights were not different between control and Se-treated rats, the offspring of Se-treated rats exhibited retarded growth between 7 and 12 wk of age, even though they were fed control diets without added sodium selenite.

Kaur and Parshad (1994) fed wild caught house rats (*Rattus rattus* L.) diets containing 2 and 4 ppm Se (sodium selenite) and found dose-dependent reductions in BW, testicular and cauda epididymis weights, sperm concentration, motility, and percentage of live spermatozoa with a simultaneous increase in the percentage of morphologically abnormal spermatozoa. Dietary Se did not lead to any significant effects on sperm head and neck morphology. The abnormalities in the midpiece of the flagellum were five times and 40 times greater in rats fed 2 ppm and 4 ppm Se, respectively, compared to control rats. The percentage of spermatozoa

exhibiting multiple abnormalities was significantly higher in rats fed 4 ppm Se compared to either the control diet or the rats supplemented with 2 ppm Se. Significant variation in the in the percentage of sperm abnormalities (10.85% to 68.75%) revealed a large individual variation in response of the rats to 4 ppm dietary Se. The morphological abnormalities were characterized as bending at the proximal, middle, and distal parts of the midpiece, bifurcation in the proximal midpiece just next to the neck or bifurcation at the joining joint of the main piece. Bending of the midpiece resulted in coiling or torsion of the proximal midpiece, the incidence of which was relatively high in rats fed 4 ppm Se. The midpiece abnormalities, including bending, torsion, and coiling as well as dismantling of different fibers of the midpiece region of the flagellum, indicates that the abnormalities occurred during the late stages of spermiogenesis. This information agrees with earlier studies in mice indicating that the postmeiotic stages of spermatogenesis (spermiogenesis) are more sensitive to Se status than earlier premeiotic spermatogenic stages (Gould, 1970).

Sanchez-Gutierrez et al. (2008) evaluated the effect of Se deficiency on *in vitro* fertilization capacity and oxidative stress of mice spermatozoa. Mice were fed a Se-deficient basal diet containing 0.02 ppm Se or the basal diet supplemented with purified L-(+)-selenomethionine, to achieve a final Se concentration of 0.2 ppm. The concentration (14.7 x 10^6/mL vs. 17.0 x 10^6/mL) and motility (59% vs. 97%) of sperm was decreased for the Se-deficient group compared to the Se-sufficient group. These results were consistent with those seen by Shalini and Bansal (2005), who found decreases in sperm count and sperm motility in Se-deficient and Se-excess (1.0 ppm Se as sodium selenite) mice. Sanchez-Gutierrez et al. (2008) also found a tendency ($P = 0.06$) for sperm from the Se-deficient group to be less viable (79% vs. 95%), as determined by triptan blue exclusion, compared to Se-sufficient animals.

Nuclei of spermatozoa from the Se-deficient mice were altered, with the appearance of heterogeneous electron-dense chromatin, frequently associated with a disrupted nuclear envelope. The assessment of chromatin condensation is relevant because chromatin is completely reorganized in the late stages of spermatogenesis when histones are replaced by protamines. The percentage of oocytes fertilized with sperm from Se-deficient mice was decreased by approximately 67% in vitro. One of the most recognized products of lipid peroxidation TBARS, a marker of oxidative stress, was increased in both spermatozoa (93.13 vs. 1.52 nmol TBARS/2x10^6 sperm) and testis tissue (0.0107 vs. 0.0067 nmol TBARS/g tissue) of Se-deficient mice compared to Se-sufficient control mice, consistent with findings of Kaushal and Bansal (2007).

Kaur and Bansal (2004b) found that male mice fed a Se-deficient diet for 8 wk had reduced fertility (50%) and sired lower litter sizes (2.0 pups/litter) compared to mice supplemented with 0.2 ppm Se (100% fertility and 8.0 pups/litter) and 1.0 ppm Se (100% fertility and 6.25 pups/litter). A subsequent study by Shalini and Bansal (2007a) investigated fertility of male mice fed a Se-deficient yeast-based basal diet compared to mice supplemented with 0.2 or 1.0 ppm Se as sodium selenite. Four weeks after the start of the dietary treatments, the Se-deficient mice exhibited 72.7% fertility compared to 88.9% fertility seen in mice fed 0.2 and 1.0 ppm Se. No effect was seen on litter size. At 8 weeks after the start of dietary treatments, however, the unsupplemented mice exhibited 41.7% fertility and the mice fed 1.0 ppm Se exhibited 44.4% fertility compared to the mice fed 0.2 ppm Se which exhibited 88.9% fertility. Litter sizes were also reduced with the deficient animals giving birth to 3.5 pups per litter, the mice fed 1.0 ppm Se giving birth to 4.6 pups per litter, and mice receiving 0.2 ppm Se giving birth to 7.0 pups per litter. A significant decrease in Se levels and glutathione peroxidase

activities were seen within the testes of deficient mice, whereas the mice receiving 1.0 ppm Se exhibited increased testis Se and glutathione peroxidase levels compared to mice fed 0.2 ppm Se. Additionally, a significant increase in lipid peroxidation, again an indicator of oxidative stress, was seen in both the Se-deficient and Se-excess group.

A recent study by Jana et al. (2008) found that coadministration of sodium selenite (6 ppm) and zinc sulfate (3 ppm) protected Wistar male rats against exercise-induced testicular spermatogenetic disorders, prevented testicular oxidative stress, and increased testicular antioxidant status. The treatments (n = 12/treatment) consisted of an unexercised control group, an exercised control group, an unexercised group of supplemented rats, and an exercised group of supplemented rats that swam together in a water tank for 4 h per d for 6 d per wk over 10-wk. When female rats were mated with male rats from the experiment, fertility rates of unexercised male rats were greater (90.9% and 91.7% pregnancy rates for control and supplemented rats, respectively) than the exercised male rats (50% and 70% pregnancy rates for control and supplemented rats, respectively). There were no indications whether the differences in pregnancy rates were statistically significant. The authors also reported that vaginal smears from females mated with unexercised control rats, unexercised supplemented rats, and exercised supplemented rats had huge numbers of sperm in each microscopic field compared to exercised control rats which had fewer sperm in each field, although no data was presented. Females were examined via laparotomy 10 d post-coitus which showed greater numbers of implantation sites in each uterine horn in female rats mated with unexercised male rats (7.0 and 8.5 for control and supplemented rats, respectively) compared to exercised male rats (4.0 and 6.0 for control and supplemented rats, respectively). There were no indications whether the differences in pregnancy rates and the number of implantation sites per uterine horn seen were statistically

significant. It is also important to note that the effects of selenium and zinc could not be separated, and that the Se levels fed in this experiment were much greater that 0.2 ppm required according to the NRC (NRC, 1995).

Olson et al. (2005) maintained Sprague-Dawley rats on a torula yeast based Se-deficient diet, or the same base diet supplemented with 0.25 ppm sodium selenate for five and seven mo. Rats fed the Se-deficient diet for five and seven mo showed similar morphological abnormalities. Plasma and liver GPx levels of the Se-deficient groups (at 5- and 7-mo) were less than 1% of those seen in the control rats, establishing that the rats fed the Se-deficient diet were severely Se-deficient. The Se-deficient rats exhibited sperm with an array of flagellar defects that arose during spermiogenesis and epididymal maturation. A comparison of late spermatids revealed that Se-deficient spermatozoa exhibited normal structural organization of the flagellar plasma membrane, axoneme, outer dense fibers, fibrous sheath, and annulus, but they exhibited an abrupt narrowing of the spermatozoon posterior midpiece due to premature termination of the mitochondrial sheath. Caput epididymal spermatozoa from control animals appeared normal at five and seven mo, whereas spermatozoa from Se-deficient rats exhibited an abrupt narrowing of the posterior midpiece, resulting from the premature termination of the mitochondrial sheath. Gaps in the proximal regions of mitochondrial sheath of Se-deficient rats were only occasionally observed. Other morphological abnormalities occasionally seen were an extrusion of a flagellar fiber from the neck region and the presence of kinks at the head-tail junction of spermatozoa from Se-deficient rats. Cauda epididymal spermatozoa from Se-deficient rats exhibited ultrastructural defects in both the flagellar principal piece and midpiece. Although the sheath appeared normal and the plasma membrane was typically present, many flagellar profiles lacked a full complement of nine axonemal doublet microtubules and their associated outer dense fibers.

Doublet microtubule – outer dense fiber complexes 8, 9, 1, 2, and 3 were present, as was as the central pair of microtubules within the fibrous sheath lumen, and all retained their normal geometric relationships. However, various combinations of doublet microtubule-outer dense fiber complex 4 to 7 were absent in Se-deficient rats. Compared to the proximal midpiece of spermatozoa from Se-deficient rats, a more frequent absence and greater disorganization of flagellar fibers were seen in the distal midpiece segment of the flagellum that lacked the mitochondrial sheath. Furthermore, spermatozoa tails from Se-deficient rats had an annulus that occurred in the proper position at the junction of the midpiece and the principal piece and was adherent to the proximal end of the fibrous sheath, but not the mitochondrial sheath.

Wu et al. (1973) investigated whether feeding vitamin E at generally recommended or highly elevated levels in the diet could modify the effects of Se deficiency on testicular function in rats. Supplementing the vitamin E and Se-deficient basal diet with 60 ppm D-α-tocopherol and 0.1 ppm Se (source unknown) led to histological active spermatogenesis within the testis, numerous sperm in the lumen of the epididymis, spermatozoa exhibiting 97% normal morphology, and a excellent motility score of 10 (on a 0 to 10 scale). Supplementing the basal diet with only 60 ppm or 1000 ppm D-α-tocopherol led to histological active spermatogenesis within the testis and numerous sperm in the lumen of the epididymis, however the spermatozoa exhibited 22% normal morphology, and a poor motility score of 0 . Supplementing the basal diet with a different vitamin E product they referred to as "Formula S2" at 1000 ppm D-α-tocopherol led to histological active spermatogenesis in only some of the seminiferous tubules within the testis and numerous sperm in the lumen of the epididymis, however the spermatozoa exhibited 7% normal morphology, and a poor motility score of 0 . Supplementing rats with 60 ppm of the Formula S2 resulted in only one rat having active spermatogenesis and they did not get a semen

sample to analyze for morphology and motility. Rats receiving Se-deficient diets all showed similar morphological abnormalities consisting of breakage of the tail of the spermatozoa near the middle piece or principle piece of the tail. They also tested four additional antioxidants added to the basal diet, including 0.01% *N,N'*-Diphenyl-D-phenylenediamine, 0.5% ascorbic acid, 0.1% butylated hydroxytoluene, and 0.25% methylene blue, but most of the rats failed to produce motile spermatozoa. The authors concluded that the role of Se on spermatogenesis is specific and cannot be substituted by vitamin E, even at highly elevated levels.

Dimitrov et al. (2007) fed turkey toms one of two diets, a basal commercial diet that contained 0.1 ppm sodium selenite, or the basal diet plus 0.3 ppm organic Se. After 6 h of semen storage, sperm motility decreased from 71.5% to 65% in toms fed the basal diet compared to motility not changing significantly in the organic Se-supplemented group. The positive effect of organic Se supplementation on sperm motility was associated with increased concentrations of total lipids and phospholipids in the seminal plasma of the supplemented group. After the stored semen was inseminated into hens, the organic Se-supplemented group had a 90.5% fertilization rate compared to 88% for the control group of toms, but it was not indicated if this difference was statistically significant.

Boar reproductive organ development. Marin-Guzman et al. (1997) found no differences in testis measurements, including testis circumference, length, width, and weight, in control boars or boars fed a diet supplemented with 0.5 ppm sodium selenite. Similar findings were reported in other experiments in which boars were fed 0.5 ppm sodium selenite (Kolodziej and Jacyno, 2005) or injected with 0.33 ppm sodium selenite every 14 d for a 120-d period (Segerson et al., 1981). Jacyno et al. (2002) reported similar testicular volumes in boars fed diets supplemented with either 0.2 ppm inorganic Se and 30 IU vitamin E or 0.2 ppm organic Se and

60 IU vitamin E. The effects of Se supplementation on sizes of accessory sex glands have not been reported.

Studies with Se-deficient mice and rats showed similar effects, with no differences in testicular mass or morphology observed from 5 wk up to an entire yr during which Se-deficient diets were fed (Watanabe and Endo, 1991; Behne et al., 1996). However, Behne et al. (1996) reported that rats from the second Se-depleted generation on exhibited decreased testis mass, expressed as a percentage of body mass, compared to Se-adequate controls. Testis size in the fourth Se-depleted generation was less than 50% of Se-adequate control rats. In addition to the testis exhibiting severe bilateral atrophy, seminiferous tubules had substantial reductions in diameter and were almost entirely lined by Sertoli cells and a few stem cells. Differentiated spermatozoa could not be detected. These alterations, however, were reversible and spermatogenesis was restored when a Se-adequate diet was fed (Behne et al., 1996). Similar research with successive generations of Se-deficient boars has not been conducted.

Effects of Se supplementation on boar spermatogenesis. Marin-Guzman et al. (2000a) conducted an experiment employing a 2x2 factorial arrangement of treatments with 5.4 to 9 mo old boars fed dietary Se (0 or 0.5 ppm) as the first factor and vitamin E (0 or 220 IU/kg diet) was the second factor. At 5.4 or 6.2 mo of age, testicular sperm reserves were not affected by dietary Se, but at 9 months of age there was a trend for a higher number of sperm reserves in Se-supplemented boars, and by 18 mo of age the Se-fed boars had higher numbers of sperm reserves. Boars fed dietary Se had a greater number of Sertoli cells and round spermatids at 6.2 mo of age, but by 18 mo of age the boars fed Se had more Sertoli cells, more secondary spermatocytes, and more round spermatids. Vitamin E did not affect testicular sperm reserves, Sertoli cells, or germ cell populations at any age.

Effects of Se supplementation on boar semen characteristics. Segerson et al. (1981) conducted an experiment during which crossbred boars were fed a low Se diet (0.025 ppm) made of cornstarch and torula yeast on an ad libitum basis beginning at 78 d of age. Boars received s.c. injections of sodium selenite (0.33 mg/kg BW) or 0.9% saline at 14-d intervals. At 230 d of age, boars were exposed to estrogen-treated, ovariectomized gilts and ejaculates were collected at four- to six-d intervals until a total of four ejaculates had been obtained from each boar. Injections of Se increased the number of spermatozoa per ejaculate but had no effects on the percentages of viable or morphologically normal spermatozoa.

Marin-Guzman et al. (1997) fed boars a basal diet, the basal diet supplemented with Se (0.5 ppm sodium selenite), the basal diet supplemented with Vitamin E, or the basal diet supplemented with both Se (0.5 ppm) and Vitamin E. Boars fed the basal diet displayed decreased sperm motility and an increase in the percentage of sperm cells with abnormal morphology compared with the supplemented groups. The effects of added Se on semen characteristics were more pronounced than the effects of added Vitamin E, and Se supplementation resulted in greater fertilization rates with a higher number of accessory sperm in fertilized oocytes when gilts were bred with semen from the experimental boars.

Jacyno et al. (2002) compared organic versus inorganic Se supplementation on semen characteristics. Boars were fed diets supplemented with either 0.2 ppm Se-yeast and 60 ppm vitamin E, or 0.2 ppm sodium selenite and 30 ppm vitamin E. There was no effect of treatment on ejaculate volume or the percentage of motile spermatozoa. In contrast, sperm concentration and total spermatozoa were higher in boars fed the diet containing the organic source of Se. The quality of spermatozoa was also enhanced in boars receiving the organic Se source. Indeed, boars fed the diet containing organic Se had higher percentages of spermatozoa with normal

acrosomes and that passed a hypo-osmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities. Although these data are consistent with the notion that supplementation of diets with an organic source of Se is superior to supplementation with inorganic Se, source of Se in this study was confounded with dietary concentration of vitamin E.

Kolodziej and Jacyno (2005) reported that increasing Se from 0.2 ppm Se to 0.5 ppm Se increased sperm concentration and total numbers of spermatozoa, although the form of Se fed to boars was not mentioned. They also showed that the numbers of spermatozoa with major and minor morphological abnormalities and spermatozoa with defective acrosomal membranes were 3.5%, 4.8%, and 6% lower, respectively, when boars received 0.5 ppm Se. Sperm motility was not impacted by Se feeding level. The values of osmotic resistance tests were 13% higher when boars were fed 0.5 ppm Se.

Marin-Guzman et al. (2000b) conducted an experiment with 5.4 to 9 mo old boars. A 2x2 factorial arrangement of treatments was employed with dietary Se (0 or 0.5 ppm) as the first factor and vitamin E (0 or 220 IU/kg diet) as the second factor. When spermatozoa of boars were examined using electron microscopy, boars fed the low-Se diet had normal acrosome and nuclei of the spermatozoa, but the mitochondria in the tail piece were more oval with wider gaps between the organelles. The plasma membrane connection to the tail midpiece of boars fed the low-Se diet was not as tightly bound as when boars were fed Se. Immature spermatozoa with cytoplasmic droplets were more numerous when boars were fed the low-Se diet, but the occurrence of midpiece abnormalities occurred in boars with or without Se or vitamin E. Unsupplemented boars exhibited reductions in the concentration of ATP in spermatozoa and vitamin E had no effect on ATP concentration. Selenium supplemented boars had 25% greater

ATP concentrations compared to the unsupplemented boars. This response was consistent with the 27% greater sperm motility reported earlier (Marin-Guzman et al., 1997) when Se was added to boar diets. Dietary Se appears to affect the metabolic activity of spermatozoa, and thus could contribute to improvements in motility and subsequent fertilization rates in the female, consistent with previous findings (Marin-Guzman et al., 1997).

Effects of Se supplementation on boar libido. A paucity of information exists regarding the effect of Se supplementation on boar libido. Henson et al. (1983) fed crossbred boars a basal corn-soybean meal diet containing 0.05 ppm Se supplemented with sodium selenite at concentrations of 0, 0.10, and 0.25 ppm. Libido was subjectively scored upon exposure to ovariectomized, estrogen-treated gilts. While treatment alone failed to affect libido scores, there was a treatment by age interaction with the group receiving the 0.3 ppm Se diet having reduced libido scores compared to the other treatments as age increased. Kolodziej and Jacyno (2005) reported no differences in libido as Se was increased from 0.2 ppm to 0.5 ppm, although they did not report the form of Se fed.

VI. Effect of dietary selenium supplementation on gene expression

Compared to the number of gene expression studies published in mice (Rao et al., 2001; Shalini and Bansal, 2005, 2007a,b; Hooven et al., 2006), relatively few studies have been published for swine (Lei et al., 1997, 1998; Zhou et al., 2009) fed various sources of Se. Rao et al. (2001) examined gene expression profiles of mice fed either a Se-deficient basal diet (< 0.01 ppm Se) or a high Se diet, composed of the basal diet supplemented with 1.0 ppm purified seleno-L-methionine for 90 d. Genes exhibiting a greater than two-fold increase in expression in Se-deficient mice were consistent with a state of DNA damage, genetic instability, and oxidative stress, some of which included cell cycle arrest/DNA inducible genes and molecular chaperone

heat-shock proteins (HSP27 and HSP40). Genes displaying a greater than two-fold decrease in expression in response to Se-deficiency included two selenoproteins, GPx1 and D1, which exhibited 3.0- and 2.4-fold decreases, respectively. This corresponded to a 66% reduction in mRNA levels of GPx1 and a 59% reduction in mRNA levels for D1. Other selenoproteins such as selenoprotein P, GPx2 and GPx4 did not exhibit changes in gene expression within the jejunum due to Se supplementation.

Hooven et al. (2006) investigated gene expression in female mice from the third Se-depleted generation given water containing no additional Se or 0.1 or 1.0 ppm Se as sodium selenate. DNA microarrays were used to compare gene expression in leg muscle from these mice. The gene expression changes could be classified as transcription and signal transduction factors, including several genes with oncogenic potential. Genes affected were T-cell receptor beta, a T-cell-specific transcription factor, lymphocyte protein tyrosine kinase, prostaglandin E receptor, SP100 nuclear dot gene, Nfic, a nuclear factor, and Vav2, an oncogene. The most prominent expression changes were related to T-cell signal transduction, suggesting that Se status alone could affect T-cell related factors in muscle or other tissues.

Nuclear factor kappa B (NFκB), a well known redox regulated transcription factor, has also been suspected to play a crucial role in spermatogenesis. As reviewed by Delfino et al. (2003), testosterone effects on Sertoli cells for the completion of spermatogenesis, are mediated via intracellular cytosolic androgen receptors (AR) within the Sertoli cell. The hormone-bound AR complexes dimerize and translocates to the nucleus, where they bind to androgen response elements within gene promoters. A number of studies have shown that AR localization within Sertoli cell was specific to certain spermatogenic stages. Androgen receptor localization within the Sertoli cell progressively increases from stage II through stage VII of spermatogenesis,

subsequently declines during stage VIII to be barely detectable in stages IX to XIII of the 14-stage spermatogenic cycle in rats (Bremner et al., 1994; Vornberger et al., 1994). Zhang et al. (2004) determined that it is NFκB that regulates transcription of AR in primary Sertoli cell cultures isolated from adult rat testes. Delfino et al. (2003) found that tumor necrosis factor-alpha (TNFα), a secretory product of round spermatids, stimulated NFκB binding to the AR promoter, induced AR promoter activity, and increased endogenous AR expression in primary Sertoli cell cultures.

Shalini and Bansal (2007a) investigated fertility of mice fed a Se-deficient yeast-based basal diet compared to mice supplemented with 0.2 or 1.0 ppm Se as sodium selenite. Fertility of mice fed the Se-deficient and Se-excess diet (1.0 ppm) was decreased compared to mice receiving the Se-adequate (0.2 ppm Se) diet. Expression of *p65* and *p50* genes, two subunits of NFκB, increased in the testis of Se-deficient mice while the expression of IκBα, an inhibitor of NFκB, declined. These results indicate that NFκB is activated during Se deficiency. Significant elevations of inducible nitric oxide synthase (NOS) and NO levels were also seen in testis samples from Se-deficient mice. Eight wk from the start of the experiment, expression of p65, but not p50, increased while IκBα decreased in Se-excess mice, but not to the levels seen in Se-deficient mice. At four wk, NO levels were reduced in Se-excess mice compared to Se-adequate and Se-deficient mice, but at eight wk, NO levels had increased beyond that of the Se-adequate mice. Inducible NOS levels were not different in Se-excess mice. Research has previously shown that enhanced NFκB and NO levels are harmful to spermatogenesis and the progression of the spermatogenic cycle.

Shalini and Bansal (2005) investigated the effect of Se status on activator protein 1 (AP1), a composite transcription factor. AP1 is a homo or hetero dimer DNA binding protein

composed of cFos and cJun. Potent AP1 sites occur in the promoter regions of several genes related to antioxidant defense and inflammation, such as γ-glutamyl cysteine synthetase (γ-GCS), the rate-limiting enzyme in *de novo* reduced glutathione (GSH) synthesis, and superoxide dismutase (Mn-SOD). As such, AP1 is sensitive to oxidants, antioxidants, and conditions which affect the redox state of cells. Alterations in testis glutathione levels and antioxidant enzymes were noted. Redox ratios (reduced glutathione/oxidized glutathione) decreased in both Se-deficient and Se-excess groups at 4 and 8 wk, indicating an increase in oxidative stress. At four weeks, Mn-SOD levels were decreased in Se-deficient mice more so than Se-excess mice, but both were significantly lower than Se-adequate mice, but the degree of change disappeared between the groups when Mn-SOD was measured at 8 wk. Eight wk after feeding diets identical to those used by Shalini and Bansal (2007a), marked decreases were noted in testis cFos and cJun expression of Se-deficient and Se-excess mice compared to Se-adequate mice receiving 0.2 ppm Se. It was also shown that GPx and Mn-SOD levels increased while γ-GCS levels decreased. However, it was not noted whether these changes in gene expression were statistically significant. Decreases in cJun and cFos in Se-deficient and Se-excess mice could be responsible for decreased germ cell number, differentiation, and reduced fertility observed at the altered Se levels.

To further elucidate the effects of excess Se supplementation, Shalini and Bansal (2007b) cultured mouse testicular cells in vitro with and without the presence of buthionine sulfoximine (BSO), a known glutathione depletor. Testicular cells cultured with 1.5 μM Se exhibited reductions in the redox ratio, where as BSO did not affect this ratio. This indicated that sodium selenite acts as a pro-oxidant at the 1.5 μM concentration that increases oxidative stress. Sodium selenite (0.5 and 1.5 μM Se) treated cells increased cFos and cJun expression, even in BSO-

treated glutathione-depleted cells. Sodium selenite (1.5 μM) also increased the expression of *p65*, but not to the degree that *p65* expression was seen in the presence of 1.5 μM Se and BSO. Previous experimental results showed that mice fed both a Se-deficient and Se-excess diet had increased lipid peroxidation, decreased GSH, and a reduction in cFos and cJun expression. They concluded that higher levels of sodium selenite, working as a pro-oxidant, generates excessive ROS, which instead of activating gene expression of cFos and cJun, actually repress these two genes.

A fourth experiment was conducted by Kaushal and Bansal (2007) with the same dietary treatments as Shalini and Bansal (2007a). They wanted to characterize gene expression of Cdc2, cyclin B1, Cdc25c, and p21, four cell cycle regulators known to control the G2/M phase checkpoint during meiosis, which is the most critical event in spermatogenesis. Protein and mRNA expression of CDC2, Cyclin B1, and Cdc25c were found to be significantly decreased in the Se-deficient and Se-excess groups. A decrease in CDC2 activity was also observed in the two groups. The expression of p21, a kinase inhibitor, was found to be elevated in the Se-deficient and Se-excess groups. The authors proposed a pathway involving modulations in the Cdc25c and p21 in response to Se-induced oxidative stress, leading to inhibition of the Cdc2/cyclin B1 complex, thereby causing G2/M phase arrest of meiosis I and abnormal spermatogenesis. While Se-induced oxidative stress induces p21 and inhibits both Cdc25c and the Cdc2/Cyclin B1 complex, it was found that Cdc25c is more susceptible to alterations by Se-induced oxidative stress than p21. The p21 subunit was found to inhibit Cdc2/Cyclin B1 through inactivation of Cdc25c.

It is important to remind readers that unlike selenomethionine, sodium selenite acts as a prooxidant. It is feasible that selenomethionine could act in the exact opposite way should its

effects on gene expression be evaluated. In this manner, selenomethionine could hypothetically aid the cell cycle regulators to enhance meiosis, protecting spermatogenesis. More research is needed to determine how animals will respond to inorganic and organic forms of equal concentrations of supplemental selenium on the molecular and genetic level.

Zhou et al. (2009) published the first paper using the pig as an experimental model for evaluating selenoprotein gene expression in endocrine tissues, citing the problems of limited availability of human samples and the small sizes of rodent samples as the biggest barrier to profiling gene expression in endocrine tissues. Prior to conducting this experiment only 7 of the 24 and 25 selenoproteins genes identified in mice and humans, respectively, had been cloned in pigs. This group cloned an additional five selenoproteins that only had expressed sequence tags at the time. High coding sequence homology (84-94%) with human genes was found for three of the five Se-dependent GPx (GPx1, GPx2, and GPx4), two of the three iodothyronine deiodinases (Dio1 and Dio3), and one of the three thioredoxin reductases (Txnrd1). Additional selenoproteins such as Sepp1 codes for selenoprotein P, which is believed to serve as a Se transport protein, Sepw1 codes for selenoprotein W found in muscle and brain, Sephs2 codes for selenophosphate synthetase 2 that forms monoselenophosphate, the Se donor for selenocysteine biosynthesis, Sepn1 codes for selenoprotein N, which is associated with muscle diseases, and Selk that codes for selenoprotein K, a redox protein.

In the feeding experiment published by Zhou et al. (2009), three groups of weanling (3 wk of age) male pigs were fed a basal diet containing 0.02 ppm Se, or the basal diet supplemented with 0.03 ppm or 3.0 ppm Se-enriched yeast (Angel Yeast; Angel Yeast Co., Ltd., Yichang, Hubei, P. R. China). Supplementation with Se led to dose-dependent increases in Se concentrations and GPx enzyme activity within the liver, testis, thyroid, and pituitary. Testis

mRNA levels of Txnrd1 and Sep15 decreased as Se supplementation increased from 0.3 ppm to 3.0 ppm. Expression of hepatic GPx1 increased with increasing Se supplementation levels. Within the tissues, GPx2, GPx4, Dio3, and Sep15 were highest in the pituitary whereas Dio1, Sepp1, Sephs2, and GPx1 were highest in the liver. Selenium supplementation did not affect mRNA expression of any selenoprotein in the thyroid or pituitary.

VII. Effect of dietary selenium supplementation on endocrine profiles.

It is unclear how the inclusion rate and/or form of Se supplementation may affect the endocrine profile of swine. Segerson et al. (1981) reported that serum testosterone levels were similar for boars that received s.c. injections of sodium selenite (0.33 ppm) or saline at 14-d intervals. Both groups of boars were fed a basal cornstarch-torula yeast diet that contained 0.025 ppm Se.

Kaur and Bansal (2004) found that male mice fed a yeast-based diet (0.02 ppm Se) had reduced serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone compared to mice supplemented with 0.2 ppm or 1.0 ppm Se as sodium selenite. Behne et al. (1996) also found reduced testosterone concentrations in 6-mo old Se-depleted rats compared to their Se-adequate counterparts. The Se-depleted rats exhibited reduced testosterone responses two h after administration of gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG).

Jana et al. (2008) studied the effect of the coadministration of sodium selenite (6 ppm) and zinc sulfate (3 ppm) supplementation on exercise-induced oxidative stress. The treatments (n = 12/treatment) consisted of an unexercised control group, an exercised control group, an unexercised group of supplemented rats, and an exercised group of supplemented rats. The exercised rats swam together in a water tank for 4 hours per day for 6 days each week over a 10

week period. Testicular levels of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD, as well as plasma testosterone, LH, and FSH were increased ($P < 0.05$) in supplemented unexercised rats compared to unexercised control rats and decreased in exercised control rats compared to unexercised control rats. The 3β-HSD enzyme is important in steroidogenesis as it catalyzes the conversion of pregnenolone to progesterone. Progesterone is converted to 17-hydroxy progesterone, which is then converted to androstenedione, and androstenedione is converted to testosterone or estradiol. No differences were found in plasma corticosterone levels between supplemented and unsupplemented animals, but there were increased levels ($P < 0.05$) in exercised animals compared to their unexercised controls. Plasma prolactin levels were not different between the supplemented and unsupplemented rats, but prolactin levels were reduced ($P < 0.05$) in exercised unsupplemented animals compared to unexercised unsupplemented levels. No statistical comparisons were evident between the exercised and supplemented rats and the unexercised control rats. It is also important to note that the effects of selenium and zinc could not be separated, and that the Se levels fed in this experiment were much greater than the 0.2 ppm required according to the NRC. In many species including boars, LH stimulates spermatogenesis and the secretion of testosterone, a steroid hormone that along with estradiol is responsible for maintenance of libido (Bancroft, 2005). The reduction in LH, FSH, and testosterone could be associated with reduced libido but sexual behavior was not evaluated in this study. The reduction in testosterone, FSH and LH seen in the unsupplemented rats may be attributed to increased production of oxygen free radicals, such as superoxide ($O2^-$) and hydroxyls (OH^-), as seen by Abou-Seif and Youssef (2001) in diabetic humans.

Although the effect of Se deficiency on thyroid hormone metabolism has been studied extensively, very little is known about the influence of dietary Se inclusion level and/or form of

Se supplementation on thyroid hormone metabolism. Arthur et al. (1990) discovered that Se-deficient rats had increased plasma thyrotropin (TSH) even though pituitary TSH concentrations were not affected by Se status. Repletion of Se (10, 200, or 1000 µg sodium selenite via a single intraperitoneal injection) restored plasma TSH concentrations to levels of Se-supplemented animals (fed 0.1 ppm sodium selenite) within five days. Selenium deficient rats also exhibited increased plasma free thyroxine (T_4) levels while triiodothyronine (T_3), T_4, and iodine (I) levels within the thyroid gland decreased. There are at least two mechanisms that could explain these results. First, increased TSH in the plasma of Se-deficient rats would increase T_3 and T_4 synthesis and release from the thyroid, which would in turn decrease thyroid T_3, T_4, and I concentrations. Secondly, decreased glutathione peroxidase levels within the thyroid, as seen in the experiment, could increase H_2O_2 production by elimination of free radicals. Any increase in H_2O_2, which is used for the activation of iodine before the reaction with tyrosine during the synthesis of thyroid hormones, could lead to increased synthesis of T_3 and T_4.

Beckett et al. (1987) reported that Se is required to convert T_4 into the more biologically active T_3 via the selenoenzyme type I 5'-deiodinase (ID-1). Supplementing rats with 0.1 ppm Se as sodium selenite decreased plasma T_4 concentrations 30% on average, while T_3 levels increased 125% on average compared to levels seen in Se-deficient rats. Selenium supplementation did not affect plasma reverse-triiodothyronine (rT_3). Production of hepatic T_3 increased 10.5-fold when T_4 was added to liver homogenates of Se-supplemented rats compared to Se-deficient rats. The addition of T_4 and cofactors (NAD, NADP, NADH, and NADPH) to liver homogenates did not affect hepatic T_3 production. The authors concluded that Se deficiency inhibits 5 or 5'-deiodinase activity, which is responsible for the conversion of T_4 into T_3, possibly by a direct role of Se in the deiodinase complex. This is consistent with the findings

that a deficiency in Se reduced the conversion of T_4 into T_3, increasing the levels of T_4 and reducing the levels of T_3 in chickens (Jianhua et al., 2000; Chang et al., 2005) and rats (Thompson et al., 1995).

In contrast to these reports, Dhingra et al. (2004) fed rats a Se-deficient yeast-based synthetic diet supplemented with 0.2 ppm and 1.0 ppm sodium selenite. Hepatic, aortic, and thyroid 5′-deiodoinase activity was greater in the Se-adequate group (receiving 0.2 ppm Se) compared to the Se-deficient group, both of which increased progressively throughout a 3-mo feeding period. In addition, 5′-deiodoinase activities were greater in the Se-excess group (receiving 1.0 ppm Se) compared to the Se-adequate group and continued to increase throughout a three-mo period. Between 5′-deiodoinase activity measurements, the liver had the greatest activities over the three month period compared to aortic and thyroid activities. Within the Se-deficient group, T_3 and T_4 levels decreased by 12% and 7%, respectively, after 2 mo and by 10% and 11%, respectively, after 3 mo of diet feeding compared to 1-mo data. Within the Se-adequate group, T_3 and T_4 levels increased by 26% and 13%, respectively, after 2 mo and by 19% and 19%, respectively, after 3 mo of diet feeding compared to 1-mo data. Within the Se-excess group, T_3 and T_4 levels increased by 28% and 20%, respectively, after 2 mo and by 86% and 50%, respectively, after 3 mo of diet feeding compared to 1-mo data. Behne et al. (1992) showed that increasing Se supplementation to 2.0 ppm Se eliminates any increases in T_3 and T_4 concentrations.

Jianhua et al. (2000) further explored the impact of Se supplementation on 5'-deiodinase activity by supplementing purified broiler chicken diets with or without iopanoic acid, a specific inhibitor of 5'-deiodinase. Their findings showed that sodium selenite improved growth rates and feed efficiencies of broiler chickens. The inclusion of iopanoic acid halted this response.

Hepatic 5'-deiodinase activity was increased by including selenite in the diets but was inhibited by iopanoic acid. Lower inclusion levels of T_3 (0.1 ppm) in the purified diets resulted in a growth promotion similar to that seen with Se supplementation, while higher inclusion levels of T_3 (0.3 ppm) depressed growth. The group also reported that Se and T_3 supplementation led to similar increases in skeletal muscle protein breakdown, indicating that increased T_3 levels led to increased muscle protein degradation. This data confirms that Se deficiency depresses growth of broilers by inhibiting hepatic 5'-deiodinase, leading to decreased plasma T_3 concentrations.

In contrast to these findings, Chadio et al. (2006) found that supplementing Se (0.2 ppm Se as sodium selenite) to the diets of lambs did not produce differences in total plasma T_3 or T_4 levels or the ratio of T_3:T_4 prior to and after a thyrotropin-releasing hormone (TRH) challenge. However, the basal diet in that study contained 0.11 ppm Se and may not have been a Se-deficient diet.

Bates et al. (2000) characterized the distribution of three iodothyronine deiodinase isoforms in a number of different tissues and at different developmental stages in rats fed Se-sufficient and Se-deficient diets. Isoforms D1 and D2 catalyze primarily outer-ring or 5'-deiodination and thus are responsible for the conversion of T_3 to T_4, whereas the D3 isoform catalyzes inner-ring or 5-deiodination of both T_4 and T_3, thereby generating inactive products of the hormones. Although serum total T_3 and T_4 were not different between the Se-adequate and Se-deficient groups, free T_3 and T_4 levels were not determined. According to Greenspan (2004), only 0.04% of total T_3 and T_4 levels are free hormones, which happen to be the fraction of T_3 and T_4 responsible for hormonal activity, as opposed to protein-bound T_3 and T_4. Se status did not change activity of the D1 isoform in the thyroid gland, D1 and D3 isoforms in the ovary and testis, D2 and D3 isoforms in the cerebrum and placenta, or D3 isoforms in uterine E9

implantation sites, which contains uterine tissues, decidual tissue, and embryos. Hepatic D1 isoforms were decreased in Se-deficient 12 d old pups, dams of those pups, and nonpregnant female rats. Skin activities of all three isoforms were reduced in 21-d old embryos, while only activities of the D1 and D3 isoforms were decreased in 12 d old pups in response to Se-deficiency. Selenium deficiency decreased the uterine D2 deiodinase activity in 30 d old rats. Selenium deficient nonpregnant female rats were the only to show decreased cerebrum D1 and brown adipose tissue D2 enzyme activities. Pituitary D1 isoform activity was reduced in 12 day old Se-deficient neonates. Isoform D2 deiodinase activity was also decreased in E9 implantation sites in response to Se deficiency.

Both excess and deficiency of Se supply has led to impaired growth. While Arthur et al. (1990) reported decreased growth hormone (GH) levels in the pituitary of rats fed a Se-deficient diet (less than 0.005 ppm Se) for up to six weeks postweaning, no significant differences were seen in plasma GH levels. Repletion of Se-deficient rats with a single intraperitoneal injections of Se (10, 200, or 1000 µg sodium selenite) restored pituitary GH levels to that seen in Se-supplemented animals within five days. Thorlacius-Ussing et al. (1988a) and Gronbaek et al. (1995) found that long-term treatment of rats with sodium selenite (3.3 ppm) in drinking water decreased growth hormone (GH), insulin-like growth factor-1 (IGF-1), and IGF binding protein-1, -2, and -3 and resulted in growth retardation. While the exact mechanisms involved are unknown, the authors suggested that Se accumulation in secretory vesicles may lead to reduced GH secretion. Removal of the excess selenite (3.0 ppm sodium selenite) from rat diets restored normal growth rates and normalized pituitary responses to growth hormone-releasing hormone within three weeks. However, serum IGF-1 levels did not recover within three weeks of Se removal (Thorlacius-Ussing et al., 1988b). High doses of GH administered to rats during excess

Se exposure also restored growth but did not affect serum IGF-1 concentrations. The increase in growth of Se-treated or control rats in response to GH administration appears to be independent of circulating IGF-1 levels. This could result from a direct effect of GH or GH-dependent paracrine secretion of growth factors, which could include IGF-1 (Thorlacius-Ussing et al., 1988a). Since all of the above mentioned studies examined the impact of sodium selenite on growth and GH secretion, more research is needed to determine whether these results could be extrapolated to organic Se supplements.

More research utilizing frequent blood sampling over a short period of time with or with hormonal challenges is needed to determine the effect of Se supplementation on endocrine profiles in swine. In addition to sexually dimorphic hormonal inputs and feedback mechanisms, recent research in mice has shown that selenoprotein expression can differ based on sex and the particular tissue of interest (Schomburg et al., 2007) which could affect endocrine profiles in a similar manner.

VIII. Possible cellular mechanisms of selenium response

Glutathione peroxidases. The Se-dependent SeCys moiety, often included in the active sites of these enzymes, is essential for the catalytic function of selenoproteins. More than 20 different selenoproteins have been identified and sequenced; the enzymes belonging to this large family are antioxidant enzymes which detoxify free radical hydroperoxides in order to protect cellular and subcellular membranes from peroxide damage. The GPx family consists of cytosolic GPx (GPx1), plasma GPx (GPx2), gastrointestinal GPx (GPx3), phospholipid hydroperoxide GPx (GPx4), and epididymal GPx (GPx5). Unlike GPx4 which exists as a monomer that contains one SeCys residue, GPx1, GPx2, and GPx3 exist as tetramers, with each subunit containing a SeCys residue. Another important selenoprotein to highlight would be

selenoprotein P (SEPP1), a transport protein that can carry 10 to 12 Se residues (Maiorino et al., 1999; Surai, 2006).

Mahan et al. (1999) reported that both serum Se content and GPx1 activity increased as dietary Se level increased. Serum GPx1 activities increased by pig age and reached a plateau when the diet contained 0.10 ppm Se for the first 60 d of the trial, then was decreased to 0.05 ppm Se 90-d into the experiment. The organic Se pigs fed 0.05 and 0.10 ppm Se had serum GPx1 activities that tended to be lower than pigs fed the inorganic Se source, but GPx1 activities in both organic and inorganic Se-fed pigs were similar at higher Se levels (0.2 and 0.3 ppm Se). This data was similar to that reported by Mahan and Parrett (1996).

Increasing the level of Se from 0.2 ppm to 0.5 ppm in boar diets did not significantly alter the Se or GPx1 activity in blood plasma, but increased Se and decreased GPx1 activity seen in seminal plasma (Kolodziej and Jacyno, 2005). It is important to note, however, that the authors did not report what form of Se was supplemented in that experiment. Marin-Guzman et al. (1997) discovered that serum, liver, and testis Se content and GPx1 activity was higher when Se was fed (0.5 ppm Se as sodium selenite). Testis GPx1 activity increased from weaning to 145 kg BW even when Se was not added to the diet. Serum GPx1 activity was lower at each measurement period when the basal diet was fed to boars, but the activity of this enzyme was lowest between 25 and 100 kg BW, followed by an increase at higher body weights. Liver GPx1 activities were also lower when Se was not supplemented, resulting in a decline from weaning to 130 kg BW, whereupon there was a rise. The authors concluded that the increased GPx1 activity in the serum and liver when boars were unsupplemented implies that the Se requirement lessened as the boars matured. The relative GPx1 activity in the testes of the unsupplemented boars was high, averaging 66% to the GPx1 of the supplemented group. In contrast, liver GPx1 activity of

unsupplemented boars was only 17% compared to the liver activity of the supplemented boars. The authors suggested that these results depict that the testis has a higher demand for retaining and utilizing bodily stores of Se compared to other tissues.

Lei et al. (1997) found that GPx1 activity did indeed change with age. Expression of GPx1 in Se-adequate boars increased in liver, kidney, testis, and pituitary between 1 d of age and 28 d of age, and then again between 28 d of age and 180 d of age. Activity of GPx1 was higher in the heart and thyroid at 180 d of age compared to either 1 or 28 days of age. The activity in muscle decreased from 1 d to 28 d and 180 d of age, neither of which was different from each other. A subsequent study (Lei et al., 1998) showed that Se supplementation (0.2 ppm sodium selenite) was required for full expression of GPx1 activities in the liver and heart and GPx1 mRNA in the liver, but no reproductive organs were included in that study.

Peltola et al. (1996) suggested that GPx1 protects Leydig cells against H_2O_2 oxidative stress arising from steroid biosynthesis, particularly arising from the P450 enzymes, within the testis of the rat. However, the fact that GPx1 knockouts have normal fertility leads one to question the influence GPx1 has on fertility (Flohe, 2007).

Phospholipid hydroperoxide glutathione peroxidase gene expression is age-dependent, Se-dependent and tissue-specific in mammals. The concentrations and specific activities of these enzymes are dependent on an adequate source of Se in a bioavailable form (Maiorino et al., 1998). Lei et al. (1998) concluded that Se supplementation (0.2 ppm sodium selenite) was required for full expression of GPx4 activities in the liver and heart and GPx4 mRNA in the liver, but did not include any reproductive organs in their studies. Concentrations of GPx4 vary greatly between different tissues. While GPx4 accounts for almost the entire Se content in mature mammalian testes, the expression of testis GPx4 does not begin until after puberty (Zini

and Schlegel, 1997; Maiorino et al., 1998). Prior to puberty, the largest GPx4 content is in the plasma (Maiorino et al., 1998; Shalini and Bansal, 2005). Lei et al. (1997) found that GPx4 activity in Se-adequate boars at 180 days of age was higher in liver, kidney, muscle, testis, and thyroid compared to boars at 1 or 28 days of age. Pituitary GPx4 activity was not different at the different ages. Maiorino et al. (1998) investigated the role of puberty further and found that GPx4 expression in the testis does not result from direct transcriptional gene activation by testosterone, but is due to differentiation stage-specific expression in late spermatids, which are under the control of Leydig cell-derived testosterone. In studies of Se-deficient animals, Se retention was highest in the male reproductive organs, indicating there may be a homeostatic mechanism helping to spare Se loss in the male reproductive tract (Nam et al., 1998a).

The expression of GPx4 has also been shown to be stage-specific within the spermatogenic cycle. While GPx4 is transformed into an inactive protein in mature sperm that is a constituent of the mitochondrial capsule of the spermatozoan midpiece, it is abundantly expressed as an active peroxidase in spermatids (Foresta et al., 2002). Mori et al. (2001) found that GPx4 was not expressed in spermatogonia which are the cells that line the basement membrane of seminiferous tubule. Marked expression of GPx4 began in stage seven pachytene spermatogonia and continued through stage 12 spermatids, at which time expression decreased until it completely diminished at stage 18. Maximal expression was seen in stage nine and ten spermatids. These findings show that GPx4 gene expression is marked during spermiogenesis, the portion of spermatogenetic cycle in which a spermatid transforms into a mature spermatozoan. This experiment confirms results previously reported by Maiorino et al. (1998) and Nam et al. (1997a,b, 1998a) in rat, mouse, and hamster testes, respectively.

The exact function of SEPP1 remains a mystery. However, it has been proposed that SEPP1 performs as both an antioxidant and a Se transport protein, since it accounts for approximately half of the Se in plasma (Hill et al., 2003). Research has shown that SEPP1 is a monomeric protein that contains 10 to 12 molecules of Se per molecule (Allan et al., 1999). Koga et al. (1998) investigated the expression of SEPP1 mRNA in the rat testis. Northern blot analysis and in situ hybridization showed that SEPP1 was exclusively expressed in the interstitial Leydig cell fraction and was closely related to the degeneration and regeneration of the Leydig cells.

In mice, selenoprotein P expression has been shown to be sexually dimorphic in a tissue-dependent manner. At one year of age, SEPP1 mRNA concentrations are higher in livers of female mice compared to male mice. The opposite relationship occurs in the kidney, with male mice having a higher amount of SEPP1 mRNA compared to females. These results were verified with both northern blot and quantitative real-time polymerase chain reaction techniques. This does not hold true for hepatic GPx1 or renal GPx1 or GPx3, which do not differ between the sexes at one year of age (Schomburg et al., 2007). In an earlier experiment, Riese et al. (2006) found that castrating male mice decreased kidney SEPP1 mRNA levels compared to sham-operated male mice. Selenoprotein P mRNA levels were not different in ovariectomized female mice compared to ovariectomized mice treated with estradiol; no sham operated controls were used to compare these effects, however. The authors concluded that androgens must have modulatory effects on SEPP1 expression since orchidectomy alleviated the SEPP1 mRNA levels.

Olson et al. (2005) conducted an experiment to examine the impact of Se supplementation on spermatozoa in the seminiferous tubule and the capacity of these spermatozoa to synthesize selenoproteins using infertile SEPP1 gene knockout ($SEPP1^{-/-}$) and

fertile wild-type ($SEPP1^{+/+}$) male mice. Mature spermatozoa from the $SEPP1^{-/-}$ mice displayed a specific set of flagellar defects that developed temporally during spermiogenesis and after maturation in the epididymis resulting in infertility. This data was confirmed by Hill et al. (2003), who found that SEPP1 is required for sperm development and that the activity is dependent on the Se status of animals The sperm defects seen in $SEPP1^{-/-}$ mice appeared to be the same as those seen in the $SEPP1^{+/+}$ mice fed a Se-deficient diet. Selenium supplementation to $SEPP1^{-/-}$ neither reverses the development of sperm defects, nor restores fertility.

Fertility studies with four male $SEPP1^{-/-}$ mice and four male $SEPP1^{+/+}$ mice demonstrated that the SEPP1 deletion drastically reduces male fertility. The four knockout mice averaged 5.75 matings but only achieved 0.5 pregnancies, 0.25 litters delivered, and one single pup born that died prior to weaning. On the other hand, the wild type mice averaged 5 matings, 3 pregnancies, 2.75 litters born averaging 20 pups born, of which 18.25 pups survived to weaning. Hill et al. (2003) found similar problems when breeding SEPP1 knockout mice.

IX. Summary and potential for research

Selenium has been shown to be an essential trace mineral that is important in reproduction. Current FDA legislation limits supplementation of Se in swine to 0.3 ppm. Research has shown that organic Se sources, as opposed to inorganic Se sources, are more bioavailable, decrease total Se excretion, and lead to greater tissue retention. Although research has shown that Se supplementation does not affect growth or carcass characteristics of swine, there has been research showing that Se supplementation can improve semen quality of boars used for artificial insemination. More research is needed to determine the exact molecular mechanisms for the effects seen in male reproduction. Researchers believe that the improvement is based on the activity of GPx4, but this has not been definitively proven at this point. More

research is needed to further understand the impact of Se supplementation on the endocrine system and systemic gene expression in the pig, in addition to elucidating the mechanism behind the beneficial effect of Se on reproduction.

CHAPTER THREE

Running head: Growth and physiology in boars fed organic selenium

Effects of organic selenium supplementation on growth performance, carcass measurements, tissue selenium concentrations, characteristics of reproductive organs, and testis gene expression profiles in boars

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ABSTRACT: The objective was to compare growth and physiological responses in boars fed diets supplemented with organic or inorganic selenium (Se). At weaning, crossbred boars (n = 117; 8.3 kg BW) were placed in nursery pens (three boars/pen) and assigned from within blocks based on BW to receive on an ad libitum basis one of three dietary treatments: I. basal diets with no supplemental Se (controls), II. basal diets supplemented with 0.3 ppm organic Se (Sel-Plex; Alltech, Inc., Nicholasville, KY), and, III. basal diets supplemented with 0.3 ppm sodium selenite (n = 13 pens/treatment). Average daily gain (470 g/d), ADFI (896 g/d) and G:F (0.54) were similar ($P > 0.1$) among groups. Blood Se concentrations (ppb) were greater ($P < 0.01$) for boars consuming Sel-Plex (107.5 $\pm$ 4.8) or selenite (114.7 $\pm$ 4.8) compared to controls (28.4 $\pm$ 4.8). Boars were moved to a grow-finish barn and continued to receive appropriate diets on an ad libitum basis (n = 11 pens/treatment). Average daily gain (1045 g/d) and ADFI (2716 g/d) were similar ($P > 0.1$) among groups. Gain:feed was affected by treatment ($P = 0.02$) and was greater ($P < 0.06$) for boars fed Sel-Plex (0.378 $\pm$ 0.004) compared with boars fed selenite (0.368 $\pm$ 0.004) or controls (0.363 $\pm$ 0.004). Blood Se (ppb) was greater ($P < 0.01$) in growing-finishing boars consuming Sel-Plex (198.9 $\pm$ 5.5) than boars consuming selenite (171.4 $\pm$ 5.4) or controls (26.7 $\pm$ 5.4). Treatment did not affect ($P > 0.23$) HCW, dressing percent, carcass length, LM area, standardized fat-free lean, lean %, backfat thickness, visual color, firmness, marbling, or Minolta loin color scores. Selenium supplementation did not affect ($P \geq 0.17$) testis or accessory sex gland sizes. Concentrations of Se in loin, liver, kidney, testis, cauda epididymis, and accessory sex glands were greatest ($P < 0.01$) in boars receiving Sel-Plex, intermediate in boars receiving selenite, and lowest in unsupplemented boars. Microarray analysis of testis gene expression did not result in differences ($P > 0.05$) due to dietary treatment. Testis gene expression of glutathione peroxidase 4, as determined using quantitative PCR, was increased (*P*

< 0.01) in Sel-Plex boars compared to selenite boars. In summary, dietary supplementation with Sel-Plex failed to alter ADG or ADFI but enhanced G:F in grow/finish boars. More research is needed to discern the mechanism of how Sel-Plex improves feed conversion efficiency in boars.

Key words: boar, carcass, growth, selenium

INTRODUCTION

Selenium (Se) is an essential trace mineral that has important roles in many biochemical and physiological processes. Both deficiency and excess of dietary Se can lead to impaired growth and reproductive problems in males and females (Kohrle et al., 2005). In addition to the antioxidant properties of Se, it is incorporated into selenocysteine, a key component of glutathione peroxidase enzymes that protect against oxidative stress. The expression and activities of glutathione peroxidase enzymes are dependent on an adequate source of Se in a bioavailable form (Lei et al., 1998).

Because many areas of the U.S., including the southeast, produce Se-deficient grains (Mahan et al., 2005), swine diets are typically supplemented with an inorganic source of Se, sodium selenite, at a rate of 0.3 ppm, the maximum level allowed by the FDA (FDA, 2003). Mahan and Kim (1996), however, suggested that selenite may not be as biologically effective as the Se indigenous in grains, which is primarily in the organic form of selenomethionine. Mahan and Parrett (1996) found that total Se excretion decreased and tissue retention increased when an organic, rather than an inorganic, source of Se was fed.

Subsequent studies have shown that supplementing selenite (0.5 ppm) increased numbers of Sertoli cells and testicular sperm reserves of boars (Marin-Guzman et al., 2000a). Marin-Guzman et al. (1997) also showed that supplementing selenite (0.5 ppm) to boar diets improved sperm motility and morphology in addition to improving fertilization rates when gilts were

inseminated with semen from Se-supplemented boars. It is our working hypothesis that supplementing boar diets with organic Se will improve boar growth and reproductive capacity compared to diets with inorganic Se or unsupplemented diets. In the experiment reported herein, we assessed growth performance, carcass characteristics, and reproductive organ characteristics of boars fed diets supplemented with organic Se.

MATERIALS AND METHODS

The experiment was conducted at the swine research facilities located at the Virginia Tech Tidewater Agricultural Research and Extension Center in Suffolk, VA between January and June, 2006. The nursery facility at the unit provided 52 identical pens (0.91 x 1.22 m) with a nipple drinker and a stainless steel feeder with 4 feeding spaces in each pen. Pen flooring was constructed of triangular metal bars over shallow manure collection pits. Controls were set to maintain a temperature of 27 ± 1°C during the first week after weaning followed by a gradual decline to 24°C by the end of the second week after weaning and 22°C by the end of the 35-d nursery period. Throughout the study, the nursery unit was continuously illuminated by fluorescent lighting. The environmentally controlled grow-finish building consisted of 1.83-m x 3.05-m or 3.66-m x 3.05-m pens with partially slatted concrete flooring. Each pen was equipped with a nipple drinker and stainless steel feeder. All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee.

Yorkshire x Landrace boars were weaned at approximately 28 days of age, blocked by BW, placed in nursery pens (three boars/pen) and randomly assigned from within a block to one of three dietary treatments: I. basal diet that met or exceeded the nutrient recommendations for growing boars (NRC, 1998) with the exception of selenium (n = 13 pens), II. basal diet supplemented with 0.3 ppm selenium from an organic source (Sel-Plex; Alltech, Inc.,

Nicholasville, KY; n = 13 pens) that consists primarily of selenomethionine, and III. basal diet supplemented with 0.3 ppm selenium from sodium selenite (Premium Selenium 270; North American Nutrition Co., Inc., Lewisburg, OH; n = 13 pens) (Table 3-1). Boars had ad libitum access to feed and water and remained in the nursery facility for five weeks. Boars were weighed weekly and pen feed consumption was measured as feed disappearance and was determined when the pigs were weighed. Feed conversion efficiency was expressed as G:F.

At the end of the nursery period, boars were moved to a grow-finish barn (three boars/ pen) and continued to receive, on an ad libitum basis, either a basal diet or the basal diet supplemented with 0.3 ppm selenium from either Sel-Plex or sodium selenite (n = 11 pens/treatment) (Table 3-2). Boars were weighed bi-weekly and at the completion of the growing-finishing phase (average BW = 137 kg), and boar growth rate and pen feed consumption and feed conversion efficiency were determined. Basal diet formulation changes occurred during the nursery and grow-finish periods similar to phase feeding programs used in the commercial swine industry.

Blood samples were collected via jugular venipuncture from one randomly selected boar per pen at the end of the nursery and grow-finish phases and stored at 4˚C for subsequent determination of whole blood Se concentrations.

Following completion of the grow-finish phase, one randomly selected boar from each pen (n = 11 boars/ treatment) was transported 787 km to the abattoir at the University of Georgia for harvest and carcass evaluation. Hot carcass weight was obtained after slaughter and just before chilling. Carcass length was measured on the left side of the carcass 24-h postmortem. The loin from the right side of the carcass was cut (24-h postmortem) between the 10th and 11th rib and the exposed LM was traced with acetate paper to determine standard LM area. Average

midline backfat was calculated as the average of first rib, last rib, and last lumbar backfat measurements. The formula for standardized fat free lean (kg) was (8.588 + (0.465 * HCW [lb]) – (21.896 * 10^{th} rib backfat thickness [in]) + (3.005 * 10^{th} rib LM area [in^2])/2.2). Percent lean was calculated by dividing standardized fat free lean by HCW multiplied by 100 (NPPC, 2000). Colorimetric and subjective pork quality scores (color, marbling, and firmness) were determined. Visual color scores were determined from a scale of 1 to 6 (1 = pale, pinkish gray to white and 6 = dark, purplish red; NPPC, 2000). Objective color measurements, including Hunter L* (luminescence), a* (redness), and b* (yellowness) values were evaluated at the 10^{th} rib with a Minolta color reader (Miniscan XE Plus; Hunter Associates Laboratory, Inc., Reston, VA). Marbling scores were determined from a continuous scale of 1 to 10 (1 = 1% intramuscular lipid and 10 = 10% intramuscular lipid; NPPC, 2000). Firmness was determined on a scale of 1 to 5 (1 = very soft and very watery and 5 = very firm; NPPC, 1991).

Wet weights of left and right testicles, seminal vesicles, prostate, and bulbourethral glands were recorded. Testis circumference was measured with a cloth measuring tape. Testis length and diameter were measured using a vernier caliper. Average length and diameter were used to calculate paired testicular volume, assuming each testicle had a prolate spherical shape. According to Young et al. (1986), the formula to determine paired testicular volume was:

Paired testicular volume = $4/3 * \pi *$ (average testis length) * (average testis diameter)^2 * 2.

Samples of the LM, liver, kidney, testis, caput epididymis, cauda epididymis, prostate gland, seminal vesicle, and bulbourethral gland were collected and stored at 4°C for subsequent determination of Se concentrations.

A sample of the left testis parenchyma was fixed in 10% neutral buffered formalin for histological preparation and subsequent determination of seminiferous tubule diameter. The

fixed parenchyma samples were embedded in paraffin, cut into thicknesses of 5 μm, mounted onto slides, and stained with hematoxylin and eosin at a commercial laboratory (Histo-Scientific Research Laboratories; Woodstock, VA). Seminiferous tubule pictures were obtained using an Olympus DP 70 camera (magnification 40x; Olympus America Inc., Melville, NY) mounted on a BX50 photomicroscope (Olympus America Inc.). Cameras were calibrated using a micrometer to ensure the proper scale, and all measurements were made using SigmaScan Pro 5 (SPSS Inc., Chicago, IL). Two diameter measurements were made and averaged for each of 100 round or nearly round seminiferous tubules per boar, with the second measurement approximately 90° of the first.

Selenium Determination

Selenium concentrations of feed, blood, and tissues were determined at the Toxicology Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, VA. Selenium concentrations were determined using hydride generation methods on an atomic absorption spectrometer (Varian SpectrAA-220FS; Walnut Creek, CA) with a vapor generation accessory. Blood and tissue Se was determined according to Ihnat and Miller (1977), while feed Se was determined according to Connolly et al. (2003).

Isolation of Total RNA. Testis parenchyma from the right testis of five randomly chosen boars per treatment was removed immediately after death, flash-frozen in liquid nitrogen, and transferred to -80˚C until subsequent microarray analysis. Total RNA was isolated from tissues by extraction with Qiazol lysis reagent (Qiagen, Valencia, CA) followed by isopropanol precipitation of the aqueous phase. The RNA was resuspended in water and immediately purified on RNEasy Mini Columns according to the kit manufacturer's procedure (Qiagen, Valencia, CA). Total RNA quality and quantity was determined by microfluidic analysis on an

Agilent 2100 Bioanalyzer (Agilent Technologies, Foster City, CA). After isolation, the total RNA was divided into two portions, one for microarray, and one for use in real-time PCR.

Microarray analysis (n = 5 boars / treatment)

Affymetrix GeneChip Porcine genome arrays were processed at Yerkes Microarray Core at Emory University in Atlanta, GA. Standard labeling of target (one round amplification), hybridization to GeneChip Porcine Genome Arrays (hybridization, washing, staining and scanning), and data extraction and analysis were carried out on an Affymetrix GeneChip Instrument System (GeneChip Scanner 3000, Fluidic Stations 450, Hybridization Oven 640 and computer workstation loaded with GeneChip Operating Software) using the manufacturer's reagents and protocols (Affymetrix, Santa Clara, CA). Target was prepared using Affymetrix's One-Cycle Eukaryotic Target Labeling Assay with 5 μg total RNA as starting material. Briefly, the RNA was reverse-transcribed into double-stranded cDNA, which was subsequently transcribed into biotin-labeled complementary RNA with simultaneous amplification. The resulting target was hybridized to GeneChip Porcine Genome Arrays.

Real-Time PCR Analysis (n = 3 boars/ treatment)

Real-time PCR was performed by the University of Georgia Functional Genomics Resource Facility and carried out using the Applied Biosystems 7900HT Sequence Detection System. Primers and probes were custom-designed, based on GenBank sequences of target genes, by Applied Biosystems (Foster City, CA; Table 3-7). Porcine 18S rRNA was amplified as an endogenous control. Total RNA (500 ng) was transcribed into cDNA in a volume of 20 μl with Applied Biosystems' High Capacity cDNA Archive Kit, which uses random primers, following the manufacturer's protocol (25˚C for 10 min then 37˚C for 2 h). The cDNA was diluted 1:20 for use in the amplification reaction. The amplification reaction consisted of 1 μl

diluted cDNA, 6.25 µl 2X TaqMan Universal PCR Master Mix, 0.625 µl 20X custom TaqMan Gene Expression Assay (forward primer, reverse primer, and 6FAM dye: MGB labeled probe), and water up to a total volume of 12.5 µl. Thermal cycling parameters were as follows: an initial denaturating step (95˚ C for 15 min), followed by 40 cycles of denaturing-annealing/elongating (95˚ C for 15 sec, then 60˚ C for 1 min) in a 384-well optical plate.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Experimental unit was the pen for the nursery and grow-finish periods and was the individual boar for carcass characteristics and tissue measurements. Growth performance, carcass and reproductive organ data were subjected to analyses of variance with treatment and block as main effects. For carcass and reproductive organ data, live body weight was used as a covariate. Individual means were compared using the PDIFF option of the GLM procedure of SAS.

For the analysis of microarray data, the output data (CEL files) were imported into GeneSpring GX 7.3.1 expression analysis software (Agilent Technologies, Foster City, CA). The robust multichip average preprocessing, which performs a global normalization, was applied within GeneSpring, followed by a normalization step (per gene: normalize to median) which divides the signal strength of each gene for one sample by the median of every measurement taken for that gene in all the samples. Gene measurements with raw data expression level under 50 and measurements flagged as absent by the GeneChip Operating Software (in the corresponding CHP files) were filtered out. Statistically significant differences between the three diet groups were determined by Welch ANOVA, using a *P*-value cutoff of 0.05 and applying the Benjamini and Hochberg False Discovery Rate multiple testing correction.

For analysis of quantitative real-time PCR data, values for Ct (threshold cycle) were determined using Applied Biosystems 7900HT Version 2.2.1 Sequence Detection Systems software, in relative quantification study mode, with thresholds set manually. Values for Ct (within-sample triplicate wells averaged) were then exported and subjected to the REST method (Pfaffl et al., 2002). Differences in gene expression were performed using a pairwise fixed allocation randomization test, utilizing the relative expression software tool (REST version 2). The REST method uses a mathematical model based on mean threshold cycle deviation between groups and takes into account measured probe efficiencies, with significance determined by a permutation test. In the model, the gene of interest is normalized to a reference gene, in this case, 18S rRNA. For calculating probe efficiencies within the REST software tool, pooled cDNA of all available samples were run in quantitative real-time PCR reactions using 1/20, 1/40, 1/60, and 1/80 dilutions of Taqman probes. Values for Ct for the experimental samples and endogenous control, along with C_t values for the probe efficiencies, were imported into the REST software tool. Replicates of each diet group were not averaged. REST determined the ratio comparing the expression of each supplemented diet to the expression of the basal diet, and in the third case, the organic to the inorganic. The ratios were corrected for varying probe efficiencies, as calculated by the software. To correct for nonlinear appearance of ratios, downregulation was reported as -1 divided by the ratio, so that positive numbers indicated upregulation, negative numbers indicated downregulation, and a ratio of 1 indicated no differential expression. Statistical significance was set at $P < 0.05$, and tendencies were considered at $P < 0.10$.

RESULTS

Nursery performance of pigs fed the control diet or diets supplemented with organic or inorganic Se is shown in Table 3-3. During the nursery phase, ADG, ADFI, and G:F were similar ($P > 0.1$). Blood selenium concentrations (ppb) were greater ($P < 0.01$) for boars consuming Sel-Plex (107.5 ± 4.8) or sodium selenite (114.7 ± 4.8) compared to controls (28.4 ± 4.8).

Growth performance for the growing-finishing phase and carcass characteristics are shown in Tables 3-4 and 3-5, respectively. Among groups, ADG and ADFI were similar ($P > 0.1$). Gain:feed was affected by treatment ($P = 0.02$) and was greater ($P < 0.06$) for boars fed Sel-Plex compared with boars fed sodium selenite or controls. At slaughter, there were no effects ($P \geq 0.15$) of treatment on hot carcass weight, dressing percent, carcass length, LM area, standardized fat-free lean, lean %, tenth rib or average midline backfat thickness, visual color, firmness, marbling, or Minolta loin color scores. There were no effects of treatment ($P \geq 0.17$) on size or wet weight of reproductive organs (Table 3-6).

Whole blood selenium concentrations (ppb) were greater ($P < 0.01$) in grow-finish boars consuming Sel-Plex (198.9 ± 5.5) than in boars consuming sodium selenite (171.4 ± 5.4) or controls (26.7 ± 5.4). Similarly, selenium concentrations were greater ($P < 0.01$) in LM, liver, kidney, testis, cauda epididymis, prostate, seminal vesicle, and bulbourethral gland samples for boars consuming Sel-Plex compared to sodium selenite, which were greater ($P < 0.01$) than the selenium concentrations of control boars (Figures 3-1 and 3-2). While selenium concentrations from the caput epididymis were not different ($P = 0.96$) between Sel-Plex and selenite-supplemented boars, both of these groups had greater ($P < 0.01$) selenium concentrations than control boars.

After applying the Benjamini and Hochberg False Discovery Rate multiple testing correction to the microarray data, no differences were found ($P > 0.05$) in gene expression among the three dietary treatments.

Overall, the results obtained from microarray were confirmed for a subset of genes except for expression of phospholipid hydroperoxidase glutathione peroxidase 4 (GPx4) in the testis which was greater ($P < 0.01$) in Sel-Plex-supplemented boars compared to selenite-supplemented boars and expression of lipoprotein lipase (LPL) which tended to be greater ($P = 0.06$) in selenite-supplemented boars compared to Sel-Plex-supplemented boars (Table 3-8). Luteinizing hormone/choriogonadotropin receptor (LHCGR), steroidogenic acute regulatory protein (StAR), testis enhanced gene transcript (TEGT), selenoprotein P, LPL, and adipocyte fatty acid binding protein 4 (AFABP) within the testis was not different when comparing Se-supplemented boars to unsupplemented boars. Expression of LHCGR, StAR, TEGT, and AFABP was not different among Sel-Plex- and selenite-supplemented groups (Table 3-8).

DISCUSSION

Selenium concentrations within plasma, serum, or whole blood are related to Se intake and are positively correlated with each other, but can vary with the form of Se fed (Ullrey, 1987). When defining blood selenium status across livestock species: less than 50 ppb is considered deficient, 50 to 80 ppb is marginal, greater than 80 ppb is normal, and greater than 1 ppm is toxic (Koller, 1981). Thus in the current investigation, control boars fed the basal diet without selenium supplementation were in fact selenium deficient, while the boars supplemented with sodium selenite and Sel-Plex had normal blood selenium levels.

In previous research, growth rates, feed intake, and feed conversion efficiencies were similar for control boars and boars provided extra selenium in the diet at 0.5 ppm (Kolodziej and

Jacyno, 2005; Marin-Guzman et al., 1997) or via s.c. injections at 14-day intervals (Segerson et al., 1981). Consistent with this data, the current experiment showed that Se supplementation did not affect growth performance of boars in the nursery phase. While growth rate was not affected by Se supplementation during the grow-finish period, feed conversion efficiency was improved by organic Se supplementation.

Jacyno et al. (2002) conducted an experiment during which boars were fed diets supplemented with either 0.2 ppm organic selenium (selenized yeast) and 60 ppm vitamin E, or 0.2 ppm inorganic selenium (sodium selenite) and 30 ppm vitamin E from 70 to 180 days of age in both summer and winter seasons. Compared with boars that received the organic source of selenium, boars receiving the inorganic selenium had higher ADG and better feed conversion efficiency, effects that were most pronounced during the winter. There were no effects of treatment on leanness or testicular size. However, interpretation of these data is difficult because source of Se was confounded with concentration of vitamin E fed.

Our finding that feed conversion efficiency was enhanced by feeding an organic Se supplement compared to feeding sodium selenite or no supplemental Se is in contrast to findings in barrows and gilts (Mahan and Parrett, 1996; Mahan et al., 1999; Mateo et al., 2007) in which no improvements in feed conversion efficiency were reported. This dichotomy is not easily explained. One could hypothesize that the organic selenium supplement could be increasing testicular steroid hormone secretion, which could lead directly or indirectly to improvements in feed efficiency. Circulating levels of testosterone were not determined in the current study, but Kaur and Bansal (2004a) found that male mice deficient in Se exhibited reduced serum levels of LH, FSH, and testosterone compared to mice fed diets containing 0.2 ppm or 1.0 ppm Se. Behne et al. (1996) found lower serum testosterone levels in 6 month old selenium-depleted rats fed

diets containing 0.002 to 0.007 ppm Se compared to control rats fed diets that contained 0.25 to 0.3 ppm sodium selenite. The Se-deficient rats also had reduced increases in testosterone secretion following a GnRH or LH challenge. While these studies did not include an organic source of selenium, it does establish the relationship between Se deficiency and testosterone secretion.

Organic Se-induced increase in testosterone secretion may be a plausible explanation for the improved feed efficiency reported herein; however, there were no significant differences among treatments in size of accessory sex glands, sizes of which are androgen dependent (Risbridger and Taylor, 2006). Furthermore, Schinckel et al. (1984) discovered significant positive correlations between percentage of seminiferous tubules undergoing active spermatogenesis ($r = 0.72$ and $r = 0.71$), and tubules with a lumen ($r = 0.72$, $r = 0.88$), and tubule diameter ($r = 0.80$ and $r = 0.90$) with testis weight and length, respectively. Circulating levels of LH were moderately but significantly positively correlated ($r = 0.45$ to 0.49) with testis weight, testis width, testis length, and seminiferous tubule diameter, however FSH or testosterone levels were not significantly correlated with any testis measurement. However, these are circulating levels of gonadotropins and steroid hormones, and do not reflect what could be happening at the level of the testis.

In addition to being associated with Se deficiency, Roveri et al. (1992) has established that GPx4 expression increases in the postpubertal rat, and in the case of hypophysectomized rats, GPx4 expression within the testis depends on androgen stimulation. It was previously thought that expression of GPx4 was age- or gonadotropin dependent by direct transcriptional control of testosterone. An experiment by Maiorino et al. (1998) revealed that expression of GPx4was instead, stimulated during the transition of round to elongated spermatids during

spermatogenesis in the rat. As such changes in systemic circulating levels of gonadotropins or steroid hormone probably would not be affected unless a human or animal was experiencing Se-deficiency or toxicity.

Future research could focus on selenium supplementation during the most critical period during which androgens stimulate prostate growth. A review by Kohrle et al. (2005) identify a likely link between Se and the incidence of prostate cancer, with Se-deficient humans and animals linked to the incidence of prostate cancer. Se has been shown to downregulate the prostate-specific antigen and androgen receptor transcripts in cell culture. According to Risbridger and Taylor (2006), androgens stimulate prostate growth in a temporal manner, with the greatest effects occurring during fetal development, puberty, and aging. In the current study, Se supplementation was started at weaning (28 d of age) and was continued until the boars were market weight, which corresponded to a peripubertal period with puberty occurring between 5 to 8 mo of age (Senger, 2003).

In contrast, Henson et al. (1983) reported that boars fed diets supplemented with selenium exhibited signs of retarded growth and sexual development. In that study, boars were fed a basal corn and soybean meal diet containing 0.05 ppm selenium or the basal diet supplemented with a selenium premix at concentrations of either 0.10 or 0.25 ppm. The authors reported a treatment by age interaction for BW, testis width and libido (subjectively scored twice weekly upon boar exposure to ovariectomized, estrogen-treated gilts). Plasma testosterone levels were greater for boars fed the basal diet than for those fed the basal diet supplemented with selenium. Thus, the mechanism by which the organic source of Se enhances feed conversion efficiency requires further study.

In general, no differences have been reported in carcass measurements among gilts and barrows fed inorganic or organic selenium supplements (Mahan and Parrett, 1996; Mahan and Peters, 2004; Mateo et al., 2007). The current report extends these findings to the boar.

Selenium supplementation did not affect boar testis measurements or weights of the accessory sex glands. This agrees with findings of Henson et al. (1983) who found no differences in testis weight, length, circumference, or sperm production in boars receiving diets containing 0.05 ppm, 0.15 ppm, or 0.3 ppm Se. However, as previously mentioned, there was a treatment by age interaction for testis width, and testosterone values were greater for boars fed the basal diet than for those fed the basal diet supplemented with Se. Kolodziej and Jacyno (2005) reported no differences in testes volume between boars (70 to 180 days of age) supplemented with 0.2 mg Se and 30 mg Vitamin E/kg diet or those supplemented with 0.5 mg Se and 60 mg Vitamin E/kg diet.

Segerson et al. (1981) demonstrated that Se injection of 0.33 mg Se/kg BW every 14 d increased Se concentrations in serum, kidney, liver, heart, skeletal muscle, testis, epididymis, prostate, seminal vesicle, and bulbourethral gland of boars. The current report expands on this research to include form of Se supplementation in diets of growing boars. Tissue Se levels were generally highest when organic Se was fed. Concentrations of Se reported herein are lower than those previously reported by Mahan and Parrett (1996) and Mahan et al. (1999), but the overall pattern is similar in kidney, liver, and loin samples, with Se concentrations being greatest in growing-finishing pigs fed 0.3 ppm organic Se, intermediate from pigs fed 0.3 ppm sodium selenite, and least from pigs fed the unsupplemented basal diet that contained 0.06 ppm Se. The variation between the Se values reported in the present study and those reported by Mahan and Parrett (1996) and Mahan et al. (1999) could be due to sex, though the previous researchers did

not identify the numbers of barrows and the numbers of gilts used in their research. Another possible explanation for the difference between the current data and previously published reports is inherent differences due to the laboratories conducting the assays (Cromwell et al., 1999), although similar methods were employed.

The gene expression data presented herein examined molecular biomarkers that could impact the health and physiology of the boar. The microarray gene expression did not show that there were any more genes differentially regulated due to selenium supplementation than those that were to be expected to be differentially regulated due to chance. However, our PCR data showed that GPx4, the primary glutathione peroxidase in the mammalian testis (Maiorino et al. 1998), was upregulated in boars fed Sel-Plex compared to those fed sodium selenite. Cytosolic glutathione peroxidase-1 (GPx-1), the classic and first GPx enzyme tested more than two decades ago, was not affected by Se supplementation. Given that Flohe (2007) showed that GPx1 mouse gene knockouts had normal fertility, one would have to question the role that GPx1 actually plays in fertility. Another selenoprotein, selenoprotein P, believed to be the primary Se transport protein (Saito and Takahashi, 2002), was not differentially regulated due to Se supplementation. There is a lack of information regarding the role of selenoprotein P in Se transport and bio-availability in the pig.

In conclusion, an organic source of selenium (Sel-Plex) failed to alter ADG gain or feed consumption in growing boars, but enhanced feed conversion efficiency during the grower-finisher phase of production. Carcass and reproductive organ characteristics were not affected by selenium supplementation. Selenium content of blood and most reproductive tissues was greatest for boars fed a diet supplemented with organic Se; intermediate for boars fed a diet

supplemented with inorganic Se, and lowest for boars fed a diet with no additional Se. The mechanisms responsible for the effects on feed conversion efficiency remain to be elucidated.

Table 3-1. Diet composition during nursery phases

	Nursery Diet Phase[a]		
Ingredient	I	II	III
Corn	46.72	50.58	65.35
Soybean meal	20.00	34.00	29.00
Dried whey	20.00	10.00	--
Fish meal	5.00	--	--
Spray dried plasma protein (Appetein)	4.00	--	--
Fat	2.50	2.50	2.50
Dicalcium phosphate	0.38	1.26	1.44
Calcium carbonate	0.71	0.91	0.96
Salt	0.25	0.25	0.35
Lysine-HCl	0.05	0.15	0.10
D,L-Methionine	0.02	--	--
Vitamin premix[b]	0.10	0.10	0.05
Choline chloride (60% choline)	0.02	0.02	0.02
Trace mineral premix[c]	0.10	0.08	0.08
Copper sulfate	0.10	0.10	0.10
Filler[d] or Se source[e]	0.05	0.05	0.05

[a] Diets were prepared by first mixing a basal diet that included ingredients common to each test diet. Individual experimental diets were then prepared by thoroughly mixing the appropriate test ingredient (filler, sodium selenite, or selenized yeast) with the appropriate quantity of basal diet. Nursery phase I diet was fed for week one of the 5-wk trial, phase II diet for week two, and phase III diet for weeks three, four, and five. Nursery phase I diets contained 0.131 ppm, 0.385 ppm, and 0.217 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex. Nursery phase II diets contained 0.052 ppm, 0.348 ppm, and 0.176 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex. Nursery phase III diets contained 0.027 ppm, 0.238 ppm, and 0.192 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex.

[b] BASF product, provides per kg of premix: 22,046,000 IU vitamin A, 2,204,600 IU cholecalciferol, 132,276 IU vitamin E, 4409 mg vitamin K as menadione, 60 mg vitamin B_{12}, 60,626 mg niacin, 55,115 mg pantothenic acid, 16,534 mg riboflavin, 716 mg biotin, 3,306 mg folic acid, 9,920 mg pyridoxine, and 3,306 mg thiamin.

[c] S-145 product (with no Se), provided per kg of premix: 133,300 mg iron sulfate, 166,700 mg zinc oxide, 66,720 mg manganese oxide, 20,000 mg copper sulfate, and 1,709 mg iodine.

[d] Filler consisted of a mixture of equal portions of ground limestone and ground corn to fill 0.05% of the diet.

[e] Premium Selenium 270 (North American Nutrition Co., Inc., Lewisburg, OH) containing 600 ppm Se as sodium selenite or Sel-Plex (Alltech Inc., Nicholasville, KY) containing 600 ppm Se as selenium yeast product.

Table 3-2. Grower and Finisher Diet composition

	Diet Phase[a]		
Ingredient	Grower I	Grower II	Finisher
Corn	71.07	76.22	82.02
Soybean meal	25.25	20.50	15.00
Fat	1.00	0.75	0.50
Dicalcium phosphate	0.95	0.80	0.80
Calcium carbonate	0.95	0.95	0.85
Salt	0.35	0.35	0.35
Lysine-HCl	0.05	0.05	0.05
Vitamin premix (BASF)[b]	0.05	0.05	0.05
Trace mineral premix (S-145; no Se)[c]	0.08	0.08	0.08
Tylan 10	0.20	0.20	0.20
Filler[d] or Se source[e]	0.05	0.05	0.05

[a] Diets were prepared by first mixing a basal diet that included ingredients common to each test diet. Individual experimental diets were then prepared by thoroughly mixing the appropriate test ingredient (filler, sodium selenite, or selenized yeast) with the appropriate quantity of basal diet; Grower phase I diets were fed for five wk of the 16-wk grow-finish trial, grower phase II diets were fed for five wk, and the finisher phase diets were fed for six wk. Grower phase I diets contained 0.029 ppm, 0.250 ppm, and 0.294 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex. Grower phase II diets contained 0.027 ppm, 0.324 ppm, and 0.290 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex. Finisher diets contained 0.024 ppm, 0.266 ppm, and 0.331 ppm Se, respectively, for diets containing no supplemental selenium, sodium selenite, and Sel-Plex.

[b] BASF product, provides per kg of premix: 22,046,000 IU vitamin A, 2,204,600 IU cholecalciferol, 132,276 IU vitamin E, 4409 mg vitamin K as menadione, 60 mg vitamin B_{12}, 60,626 mg niacin, 55,115 mg pantothenic acid, 16,534 mg riboflavin, 716 mg biotin, 3,306 mg folic acid, 9,920 mg pyridoxine, and 3,306 mg thiamin.

[c] S-145 product (with no Se), provided per kg of premix: 133,300 mg iron sulfate, 166,700 mg zinc oxide, 66,720 mg manganese oxide, 20,000 mg copper sulfate, and 1,709 mg iodine.

[d] Filler consisted of a mixture of equal portions of ground limestone and ground corn to fill 0.05% of the diet.

[e] Premium Selenium 270 (North American Nutrition Co., Inc., Lewisburg, OH) containing 600 ppm Se as sodium selenite or Sel-Plex (Alltech Inc., Nicholasville, KY) containing 600 ppm Se as selenium yeast product.

Table 3-3. Growth performance during the 5-wk nursery phase in boars fed a diet with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources.

Item:	Control	Sel-Plex	Sodium Selenite	SE	*P*
No. of pens[1]	13	13	13	---	---
Average daily gain, g	475	469	466	16	0.93
Average daily feed intake, g	900	896	892	27	0.98
G:F	0.54	0.54	0.54	0.01	0.89

[1]Each pen contained 3 boars.

Table 3-4. Growth performance during the grow-finish phase in boars fed a diet with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources.

Item:	Control	Sel-Plex	Sodium Selenite	SE	*P*
No. of pens[1]	11	11	11	---	---
ADG, g	1033	1066	1033	22	0.49
ADFI, g	2669	2696	2693	57	0.94
G:F	0.363[a]	0.378[b]	0.368[a]	0.004	0.02

[1]Each pen contained 3 boars.
[a,b]Within a row, means with different superscripts differ ($P < 0.06$).

Table 3-5. Carcass characteristics in control boars and boars fed diets with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources.

Item:	Control	Sel-Plex	Sodium Selenite	SE	*P*
No. of boars	11	11	11	---	---
Hot carcass weight, kg	97.0	97.4	97.8	0.5	0.80
Dressing %	74.5	74.9	74.9	0.4	0.73
Carcass length, cm	85.6	86.1	85.6	0.8	0.87
Loin muscle area, cm^2	58.7	56.1	59.4	1.9	0.38
SFFL[a], kg	53.3	54.9	53.2	0.7	0.24
Lean %	55.1	56.4	54.8	0.7	0.30
Backfat thickness, mm					
Tenth rib	20.5	19.7	17.2	1.4	0.23
Average midline	25.8	26.1	23.2	1.1	0.15
Visual color score	2.86	2.73	2.60	0.24	0.73
Firmness score	2.34	2.31	2.43	0.32	0.96
Marbling score	1.80	1.58	1.80	0.20	0.69
Loin color					
Minolta L*	48.28	46.47	49.99	0.42	0.68
Minolta a*	10.67	10.32	11.28	0.41	0.27
Minolta b*	16.16	16.24	16.95	0.55	0.53

[a]Standardized Fat-Free Lean

Table 3-6. Reproductive organs in control boars and boars fed diets with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources.

Item:	Control	Sel-Plex	Sodium Selenite	SE	*P*
No. of boars	11	11	11	---	---
Mean testis					
Weight[a], g	338.2	374.8	327.8	18.1	0.17
Length, mm	116.0	120.8	113.4	3.0	0.23
Circumference, mm	219.0	227.0	220.0	4.0	0.34
Diameter, mm	72.4	75.7	72.8	1.7	0.34
Paired test. vol.[b], cm^3	646.8	738.1	638.4	42.8	0.20
Sem. tubule diameter[c], µm	0.194	0.203	0.190	0.005	0.25
Accessory Sex Gland Weight[a]					
Bulbourethral glands, g	224.8	207.1	214.3	16.8	0.74
Prostate, g	11.8	10.9	11.9	1.3	0.79
Seminal vesicles, g	240.7	282.7	262.4	36.6	0.71

[a]Wet weight
[b]Paired testicular volume
[c]Seminiferous tubule diameter

Table 3-7. Primer pairs for each selected gene, probe sequences, and Genebank accession number from which the primer pairs were selected.

Gene	Probe size, bp	Probe sequence	Primer sequences	Genebank Accession number
GPx4[1]	71	AAGAGCCCCAGGTCATAG	Forward: AGAACGGCTGTGTGGTGAAG Reverse: GCACGGCAGGTCCTTCT	NM_214407
LHCGR[2]	88	TCACTCTGAGAGATTTC	Forward: CTTTCAGAGGACTTAATGAGGTCGTA Reverse: AGGTTGTCAAAGGCATTAGCTTCT	NM_214449
StAR[3]	67	CCTGCCGGCTCTCCT	Forward: CTGGACATCCTCAGCAACCA Reverse: GCACCTCGTCCCCATTCT	NM_213755
TEGT[4]	62	CCCAGCATCCTTCCC	Forward: TGGACTTGTGCATTGCCATTAAC Reverse: CATTGCCGTGCCCATGAAG	NM_001005348
Selenoprotein P	103	ACAGTGACGTTTGCCTCC	Forward: CAGAGGAGAACGTCATTGAATCTTG Reverse: CAACTTCATTTGGTGCTAGCTTCTG	EF113596
LPL[5]	95	ATCTAGGCCAGTAATTCT	Forward: TGGCATTGCAGGAAGTCTGA Reverse: GGCTTGGAGCTTCTGCATACTC	NM_214286
AFABP[6]	72	CAGGAAAGTCAAGAGCACC	Forward: GGCCAGGAATTTGATGAAGTCACT Reverse: GGCGCCTCCATCTAAGGTTAT	AF102872
18S rRNA	71	CAGCCACCCGAGATTG	Forward: AGGGCATCACAGACCTGTTATTG Reverse: CCCCAACTTCTTAGAGGGACAAG	NR_002170

[1]Glutathione Peroxidase 4 (GPx4)
[2]Luteinizing hormone/choriogonadotropin receptor (LHCGR)
[3]Steroidogenic acute regulatory protein (StAR)
[4]Testis enhanced gene transcript (TEGT)
[5]Lipoprotein lipase (LPL)
[6]Adipocyte fatty acid binding protein 4 (AFABP)

Table 3-8. Microarray data validation on a subset of genes by qPCR. Log 2-transformed Affymetrix array fold changes and qPCR expression ratios (n = 3/treatment).

	Microarray		qPCR	
Gene Symbol	Log 2 Fold Change	*P*-value	Log 2 Ratio	*P*-value
Sel-Plex supplemented boars compared to control boars				
GPx4[1]	-0.149	0.458	0.212	0.340
LHCGR[2]	0.133	0.469	-0.207	0.793
StAR[3]	0.205	0.663	0.091	0.742
TEGT[4]	-0.005	0.646	0.120	0.593
Selenoprotein P	0.337	0.475	0.302	0.513
LPL[5]	-0.155	0.559	-0.539	0.196
AFABP[6]	0.109	0.535	-0.606	0.458
Sodium selenite supplemented boars compared to control boars				
GPx4	-0.092	0.520	-0.154	0.554
LHCGR	-0.100	0.487	-0.068	0.938
StAR	0.167	0.529	-0.468	0.502
TEGT	0.055	0.625	-0.055	0.384
Selenoprotein P	0.450	0.849	0.455	0.467
LPL	0.070	0.561	0.145	0.719
AFABP	0.029	0.487	-0.997	0.137
Sel-Plex supplemented boars compared to selenite supplemented boars				
GPx4	-0.058	0.662	0.366	0.001
LHCGR	0.237	0.592	-0.139	0.879
StAR	0.037	0.490	0.559	0.429
TEGT	-0.055	0.502	0.175	0.202
Selenoprotein P	-0.112	0.602	-0.153	0.558
LPL	-0.219	0.636	-0.684	0.060
AFABP	0.080	0.477	0.391	0.530

[1]Phospholipid Hydroperoxide Glutathione Peroxidase 4 (GPx4)
[2]Luteinizing hormone/choriogonadotropin receptor (LHCGR)
[3]Steroidogenic acute regulatory protein (StAR)
[4]Testis enhanced gene transcript (TEGT)
[5]Lipoprotein lipase (LPL)
[6]Adipocyte fatty acid binding protein 4 (AFABP)

Figure 3-1. Tissue selenium concentrations (ppb wet basis) in boars (n = 11 boars/treatment) fed diets with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LSMeans ± SE. There was an effect of treatment, $P < 0.01$. [a,b,c]Within a tissue, means with different superscripts differ, $P < 0.01$.

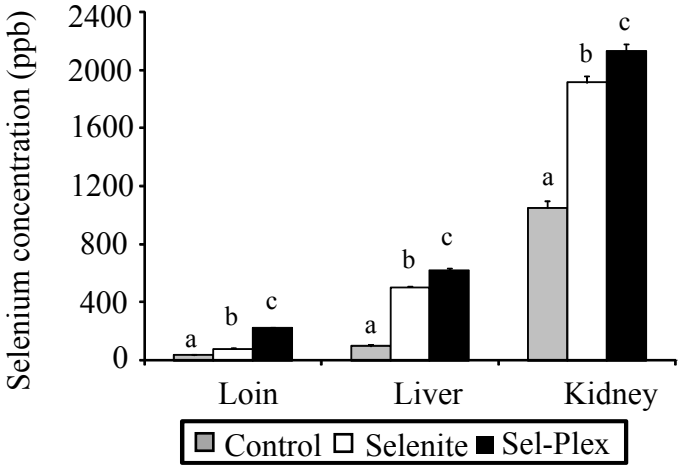
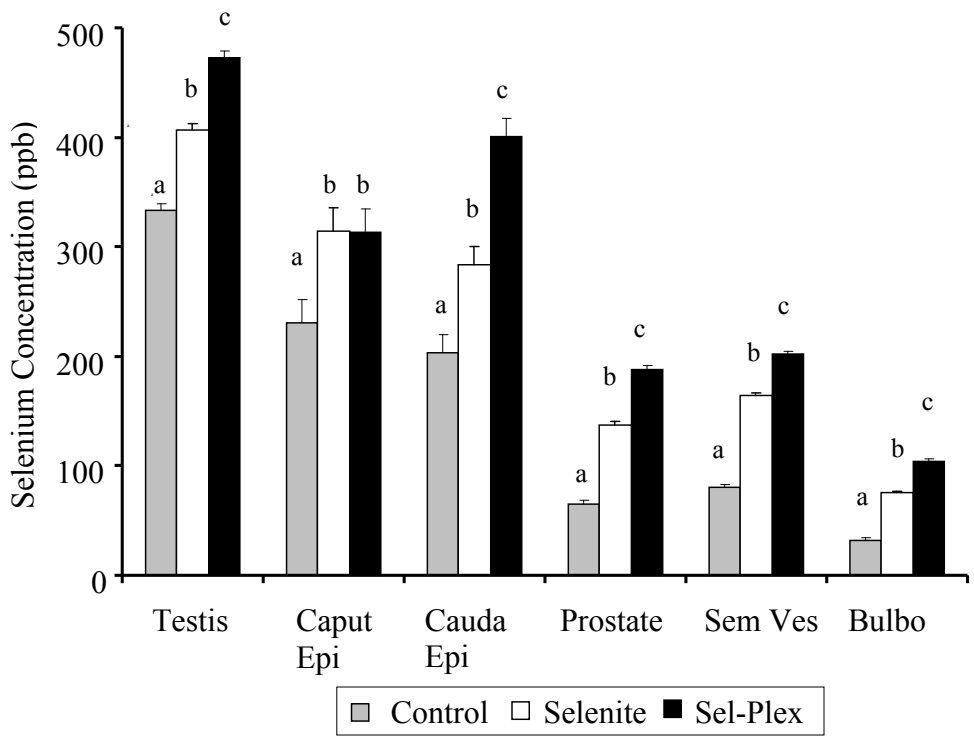

Selenium concentration (ppb)
2400
2000
1600
1200
800
400
0
a
b
c
a
b
c
a
b
c
Loin
Liver
Kidney
Control
Selenite
Sel-Plex

Figure 3-2. Tissue selenium concentrations (ppb wet basis) in boars (n = 11 boars/treatment) fed diets with no supplemental selenium (control) or diets supplemented with 0.3 ppm selenium from organic (Sel-Plex) or inorganic (sodium selenite) sources. Tissues examined include testis, caput epididymis (caput epi), cauda epididymis (cauda epi), prostate, seminal vesicles (sem ves), and the bulbourethral gland (bulbo). Values are LSMeans ± SE. There was an effect of treatment, $P < 0.01$. [a,b,c]Within a tissue, means with different superscripts differ, $P < 0.01$.


Selenium Concentration (ppb)
500
400
300
200
100
0
a
b
c
a
b
b
a
b
c
a
b
c
a
b
c
a
b
c
Testis
Caput Epi
Cauda Epi
Prostate
Sem Ves
Bulbo
Control
Selenite
Sel-Plex

CHAPTER FOUR

Running head: Semen responses to dietary organic selenium in boars

Effects of dietary organic selenium supplementation on characteristics of semen quality and in vitro fertilizing capacity of boars

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ABSTRACT: The objective was to assess semen characteristics in boars fed an organic selenium (Se) source. Crossbred boars were randomly assigned to one of three dietary treatments: I. basal diets with no supplemental Se (control), II. basal diet supplemented with 0.3 ppm organic Se (Sel-Plex), and III. basal diet supplemented with 0.3 ppm sodium selenite. At 15 mo of age, semen was collected from boars (n = 10/treatment) on 5 consecutive d. In general, semen quality decreased with time, but the negative effects of day on sperm motility were least pronounced in Sel-Plex boars. Effects of treatment x day were detected for progressively motile (P = 0.02) and rapidly moving (P = 0.03) spermatozoa, and measures of sperm velocity, including path velocity of the smoothed cell path (VAP; P = 0.05), and average velocity measured in a straight line from the beginning to the end of track (P = 0.05). At 17 mo of age, semen was collected from boars (n = 10/treatment), extended in Beltsville Thawing Solution or Androhep Lite and stored at 18˚C for 10 d. Effects of treatment x day were detected for percent motile spermatozoa (P < 0.01), static spermatozoa (P = 0.009), VAP (P = 0.06; tendency), amplitude of lateral head displacement (ALH; P = 0.02), and straightness (P = 0.01). In general, sperm cells from Sel-Plex boars were better able to maintain motility during liquid storage. At 23 mo of age, semen was collected from boars (n = 6/treatment), processed and stored in Androhep-Lite at 18° C and was evaluated at d 1 and 8 (day of semen collection = d 0) using in vitro fertilization procedures with porcine oocytes. No significant effects (P < 0.05) were seen due to treatment or day x treatment on any of the response variables. The results of this study suggest positive effects of Sel-Plex supplementation on boar semen characteristics and that dietary organic Se supplementation may help ameliorate the negative effects of semen storage on sperm motility. Dietary Se supplementation, however, did not affect in vitro fertilizing capacity of boars.

Key words: boar, fertility, selenium, semen

INTRODUCTION

The use of AI in the U.S. swine industry has increased from 8% of females bred in 1991 (Burke, 2000) to nearly 70% in 2000 (Lawrence and Grimes, 2001). The use of AI will continue to increase until practically all commercial pork producers will employ this reproductive technology. To remain efficient, however, AI programs will need to develop management and nutritional strategies for enhancing boar semen production and fertility.

Dietary selenium (Se) deficiency and toxicity can lead to impaired growth and reproductive problems in males and females (Kohrle et al., 2005). In addition to the antioxidant properties of Se, it is incorporated into selenocysteine, a component of glutathione peroxidase enzymes that protect against oxidative stress. The expression and activities of glutathione peroxidase enzymes are dependent on an adequate and bioavailable source of Se (Lei et al., 1998).

Since the southeast and many other areas in the U.S. produce Se-deficient grains (Mahan et al., 2005) swine diets are typically supplemented with sodium selenite, at a rate of 0.3 ppm, the maximum rate allowed by the U.S. Food and Drug Administration (FDA, 2003). Mahan and Kim (1996), however, suggested that inorganic sodium selenite may not be as biologically effective as the organic Se indigenous in grains, selenomethionine.

Studies have shown that diets supplemented with 0.5 ppm sodium selenite increased numbers of Sertoli cells and testicular sperm reserves of boars (Marin-Guzman et al., 2000a). Marin-Guzman et al. (1997, 2000b) also showed that supplementing 0.5 ppm sodium selenite to boar diets improved sperm motility, morphology, and fertilization rates. It is our hypothesis that supplementing boar diets with organic Se will improve reproductive characteristics compared to

boars supplemented with an equal concentration of inorganic Se or no supplemental Se. In the experiment reported herein, we assessed semen characteristics and in vitro fertilizing capacity of boars fed diets supplemented with organic Se.

MATERIALS AND METHODS

General. The experiment was conducted at Virginia Tech's Tidewater Agricultural Research and Extension Center in Suffolk, VA and all procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. Beginning at weaning (28 d of age), crossbred boars (Yorkshire x Landrace) were randomly assigned to one of three dietary treatments: I. basal diets that met or exceeded the nutrient recommendations for nursery then growing-finishing boars (NRC, 1998) with the exception of Se (control), II. basal diets supplemented with 0.3 ppm organic Se (Sel-Plex; Alltech, Inc., Nicholasville, KY), and III. basal diets supplemented with 0.3 ppm inorganic Se (sodium selenite; Premium Selenium 270; North American Nutrition Co., Inc., Lewisburg, OH). Boars were allowed water and feed on an ad libitum basis. Following completion of the grow-finish phase, the boars were limit fed at a rate of 2.5 kg/d the appropriate diets as contained in Table 4-1. Boars were housed in individual pens (4.5 m^2) that had a combination of concrete and solid steel rod flooring. At approximately seven mo of age, boars were trained to mount an artificial sow (Minitube of America, Inc.; Verona, WI) and allow semen collection. Semen was collected using the gloved hand technique.

Prior to the semen collection experiments, blood samples were collected via jugular venipuncture from each boar and stored at 4˚C for subsequent determination of whole blood Se concentrations. Blood Se was determined at the Toxicology Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, VA using hydride generation

methods on an atomic absorption spectrometer (Varian SpectrAA-220FS; Walnut Creek, CA) with a vapor generation accessory according to Ihnat and Miller (1977).

Semen Collection Challenge. At approximately 15 mo of age, semen was collected on five consecutive d from each boar (n = 10 boars/treatment) and was analyzed for volume, sperm concentration, total sperm cells, characteristics of sperm motility, and morphology as previously described (Kozink et al., 2004; Estienne et al., 2007). Briefly, semen was filtered (US BAG, Minitube of America, Inc.) during collection to remove gel. Gel-free volume and gel weight were determined gravimetrically using a top-loading balance (Acculab; Minitube of America, Inc.). Semen samples were diluted in an extender (Androhep Lite; Minitube of America, Inc.) to achieve a ratio of semen to extender of 1:5. A computer-assisted sperm analysis system (Integrated Visual Optical System, Version 12; Hamilton Thorne Research, Beverly, MA) using starting values for boar sperm analysis consistent with manufacturer recommendations, was employed to determine sperm concentration and the following characteristics of sperm motility: the percentages of spermatozoa exhibiting motility and progressive motility, path velocity of the smoothed cell path (VAP), average velocity measured in a straight line from the beginning to the end of the sperm track (VSL), average velocity measured over the actual point to point track followed by the sperm cell (VCL), straightness (STR; average value of the ratio VSL/VAP) which measured the departure of the sperm cell path from a straight line, linearity (LIN; average value of the ratio VSL/VCL) which measured the departure of the cell track from a straight line, frequency with which the sperm track crossed the sperm path (i.e., frequency of sperm head crossing the sperm average path in either direction) (BCF), and amplitude of lateral head displacement corresponding to the mean width of the head oscillation as the sperm swam (ALH). The percentage of morphologically normal sperm cells was determined after semen was eosin-

stained and dried using light microscopy (1000x). Morphological abnormalities included abnormal head, abnormal tail, detached head, bent tail, proximal droplet, distal droplet, or other. Other was defined as not meeting the criteria for the other classifications. A total of 100 spermatozoa from each ejaculate were evaluated.

Semen Storage Experiment. At approximately 17 mo of age, semen was collected from each boar (n = 10 boars/treatment) as previously described. Each ejaculate was divided into two aliquots, which were diluted in either Beltsville Thawing Solution (BTS; Minitube of America, Inc.) or Androhep Lite (Minitube of America, Inc.) to achieve a final concentration of 3 x 10^9 sperm/85 mL semen and extender. Extended semen was stored in plastic AI bottles (Minitube of America, Inc.) within a semen storage unit (Minitube of America, Inc.) at 18°C. Characteristics of sperm motility and morphology were assessed using procedures previously described. Additionally, pH was assessed daily using a pH meter (Orion model 620 pH meter; Thermo Scientific., Waltham, MA).

In Vitro Fertilizing Capacity. At approximately 25 mo of age, semen was collected from each boar (n = 6 boars/treatment) as previously described. Semen samples were diluted in an extender (Androhep Lite; Minitube of America, Inc.) to achieve a concentration of 3 x 10^9 spermatozoa/85 mL semen and extender. After dilution, semen was placed in plastic AI bottles and was shipped overnight to the Virginia Polytechnic Institute and State University in Blacksburg, VA where the bottles were stored within a semen storage unit (Minitube of America, Inc.) at 18°C for subsequent *in vitro* fertilization (IVF) experimentation.

Previously described procedures for IVF were used (Whitaker and Knight, 2004) . Unless otherwise stated, chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO). The IVF medium used was a modified Tris-buffered medium (mTBM) containing 113.1

mM NaCl, 3 mM KCl, 7.5 mM $CaCl_2$•2 H_20, 20 mM Tris crystallized free base, 11mM D(+)-glucose, 5mM sodium pyruvate, 0.4% BSA (fraction V; 43H1097, initial fraction by heat shock) and 2.5 mM caffeine according to Fan and Sun (2004) for liquid semen. Medium was filtered through a 0.22 μm pore Millipore Millex sterile syringe filter (33 mm diameter, PES membrane, Fisher Scientific, Pittsburgh, PA) and allowed to equilibrate at 39ºC in an atmosphere of 5% CO_2 for at least 12 h.

Porcine oocytes having a compact cumulus several cell layers thick were purchased from Bomed, Inc. (Madison, WI). Fifty oocytes were loaded into 2-mL vials filled with maturation media that had been equilibrated in 5% CO_2 in air at 39°C and high humidity. These vials contained a maturation media composed of a base TC199 media with Earles' salts media supplemented with porcine FSH, sodium pyruvate, gentamycin, 10% porcine follicular fluid, L-cysteine, epidermal growth factor, and insulin. Oocytes were shipped at the germinal vesicle stage in a thermostatically controlled shipping container (Minitube of America, Inc.) maintained at 39°C (Lefric Enwall, Bomed Inc, Madison, WI, personal communication). After receipt of oocytes, and at 24 h into culture, oocytes within each vial were transferred to 35 mm x 10 mm Falcon polystyrene dishes (08-757-100A; Fischer Scientific; Pittsburgh, PA) containing 250 μl of the same maturation medium minus FSH overlaid with mineral oil (ES-005-C; Millipore Corporation, Phillipsburg, NJ) and were incubated at 39ºC in an atmosphere of 5% CO_2 for an additional 24 h.

After the 48-hour incubation, cumulus cells were removed by mixing oocytes with 0.1% hyaluronidase (type IV-S from bovine testes) in mTBM media for approximately 15 sec. Oocytes were then washed three times in 100 μL drops of mTBM contained in 35 mm x 10 mm Falcon polystyrene dishes. After washing, 30 denuded oocytes were transferred using a 5 μL

Drummond micropipette (Fisher Scientific) to each well of Nunclon six well multidishes (Fisher Scientific) containing 50 μL drops of mTBM under mineral oil. A sample of semen from each boar was removed from the 17°C incubator and was warmed inside a 39°C water bath. The concentration of spermatozoa was determined (Bright-Line hemacytometer; Fisher Scientific) and then the sample was diluted with mTBM such that the final concentration was 1 x 10^6 spermatozoa/mL. Within the Nunclon multidishes, the diluted spermatozoa (50 μL) of spermatozoa were added to each well, mixed, and the oocytes and spermatozoa were co-incubated at 39ºC in an atmosphere of 5% CO_2 for an additional 18 h. The IVF procedures were conducted for each semen sample at one day and eight days post semen collection, respectively (Figure 1). Semen was gently mixed twice daily throughout the storage period.

At the end of the IVF, oocytes were mounted between 55 mm x 22 mm Corning coverslips (Fisher Scientific) and Superfrost Plus microscope slides (Fisher Scientific) supported by four columns of a mixture of petroleum jelly and paraffin (Gulf Wax) mixed in a 9:1 ratio. Corners of the coverslips were secured using Permount (Fisher Scientific). Mounted oocytes were fixed with 25% (vol/vol) acetic acid in ethanol for 48-h at room temperature. Oocytes were stained for approximately 10 min with 1% orcein (wt/vol) in 45% acetic acid in water (vol/vol). Oocytes were destained using deionized water and examined using a phase-contrast microscope. Oocytes were characterized as being penetrated by spermatozoa (swollen spermatozoon head), polyspermic (more than one swollen spermatozoon head), or undergoing male pronucleus (MPN) formation (visual identification of a MPN).

Statistical Analysis. Semen collection challenge data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary NC). Semen characteristics were subjected to analysis of variance for a repeated measures design using a model that included treatment, boar

within treatment, day and the treatment x day interaction as possible sources of variation. Treatment was tested using boar within treatment as the error term. The effect of day was tested against treatment x day. Individual means were compared using the PDIFF option of the GLM procedure of SAS. For all experiments the level of statistical significance was set at $P < 0.05$, and tendencies were considered at $P < 0.10$.

Semen storage data were subjected to analysis of variance for a repeated measures design using a model that included treatment, boar within treatment, day, extender, treatment x day, extender x day, treatment x extender, and treatment x day x extender interactions as possible sources of variation. Treatment was tested using boar within treatment as the error term. The effect of extender and extender x treatment were tested using extender x boar within treatment as the error term. Boar within treatment, day, treatment x day, extender x boar within treatment, extender by day, and extender x treatment x day were tested against the error term. Individual means were compared using the PDIFF option of the GLM procedure of SAS.

In vitro fertilization data were analyzed using the GLM procedure of SAS. In vitro fertilization parameters were subjected to analyses of variance for a repeated measures design using a model that included treatment, boar within treatment, day and treatment x day as possible sources of variation. Treatment was tested using boar within treatment as the error term. Day and treatment x day was tested against the error term. Individual means were compared using the PDIFF option of the GLM procedure of SAS. Data from one of the selenite boars was not used for d 8 because the spermatozoa did not fertilize any oocytes.

RESULTS

Whole blood Se concentrations were affected ($P < 0.01$) by treatment, with boars supplemented with Sel-Plex having the greatest blood Se levels (0.200 ± 7.1 ppm Se), boars

supplemented with sodium selenite having an intermediate blood Se level (0.182 ± 7.1 ppm Se), and control boars having the lowest blood Se levels (0.121 ± 7.1 ppm Se).

Semen characteristics in boars fed the control diet or diets supplemented with Se from the organic or inorganic sources during the semen collection challenge are shown in Table 4-2. For many traits there was an effect of day, generally reflective of a decrease in semen quality over time. The percentage of spermatozoa with abnormal heads was affected ($P = 0.02$) by treatment. The percentage of sperm cells with abnormal heads tended to be least ($P < 0.06$) in boars fed sodium selenite (0.1) and greatest in control animals (0.5), with boars receiving Sel-Plex having an intermediate value (0.4) that was not different from the other two groups (SE = 0.1).

Effects of treatment x day were detected for the percentages of progressively motile ($P = 0.02$; Figure 4-1) and rapidly moving ($P = 0.03$; Figure 4-2) spermatozoa. Measures of sperm velocity, including VAP ($P = 0.05$; Figure 4.3) and VSL ($P = 0.05$; Figure 4-4), were also affected by treatment x day, as was the size of sperm heads ($P = 0.02$; Figure 4-5). There was a tendency ($P = 0.08$) for an effect of treatment x day on VCL. In general, reductions over time in characteristic of sperm motility were less pronounced in boars fed Sel-Plex.

Semen characteristics in boars fed the control diet or diets supplemented with Se from the organic or inorganic sources during the semen storage experiment are shown in Table 4-3. There were no effects of day x extender or treatment x day x extender ($P > 0.1$), therefore data were pooled between extenders. A tendency ($P= 0.06$) for an effect of treatment x day was detected for VAP. There were effects of treatment x day for percent motile spermatozoa ($P =0.009$; Figure 4-6), percent static spermatozoa ($P = 0.009$; Figure 4-7), ALH ($P = 0.02$; Figure 4-8), and straightness ($P = 0.01$; Figure 4-9). In general, values were indicative of an enhanced ability of sperm cells from Sel-Plex-fed boars to maintain good motion characteristics during storage.

There was no effect ($P > 0.1$) of treatment or treatment x day on any of the sperm morphology characteristics. However, there was an effect of treatment x day ($P < 0.01$) for semen pH (Figure 4-10). The pH increased during storage in semen collected from control boars and boars fed a diet supplemented with selenite compared to Sel-Plex-fed boars.

During the IVF experiment, only fertilization rate tended ($P = 0.10$) to be affected by dietary treatment (Table 4-4). Averaging data from d 1 and d 8, the Sel-Plex boars exhibited 70.7 ± 1.9 % fertilization rate compared to 60.9 ± 1.9% fertilization rate for the control boars and 63.7 ± 2.1% fertilization rate for the selenite boars. Decreases were seen in all response variables as length of semen storage increased from d 1 to d 8. There tended ($P = 0.10$) to be an effect of treatment x day only with number of monospermic oocytes, and not ($P = 0.57$) when the number was represented as percent of total oocytes. The Sel-Plex (55.2 ± 2.6) and control (51.2 ± 2.6) boars exhibited a greater ($P < 0.05$) number of monospermic oocytes fertilized on d 1 compared to the selenite (43.5 ± 2.6) boars. On d 8, the boars had similar numbers of monospermic fertilized oocytes (43.0 ± 2.6, 39.0 ± 2.6, and 42.7 ± 3.1 for Sel-Plex, control, and selenite boars, respectively).

DISCUSSION

Numerous studies have shown that Se is an essential trace mineral required for the maintenance of male fertility, and a deficiency causes reduced numbers of spermatozoa, an impairment of spermatogenesis, and reductions in fertilization capacity that are reflective of both impairments in sperm motility and characteristic sperm midpiece abnormalities where the mitochondria are located (Maiorino et al., 1999).

Kaur and Bansal (2004a) reported mice fed a Se-deficient yeast-based diet (0.02 ppm Se) had significant reductions in sperm motility (58.0% vs. 90.6% and 85.5%) and total sperm

numbers in their ejaculates (0.73 x 10^6/mL vs. 2.05 x 10^6/mL and 1.73 x 10^6/mL) compared to mice supplemented with either 0.2 or 1.0 ppm Se as sodium selenite. Watanabe and Endo (1991) discovered that Se-deficient mice had increased numbers of morphologically abnormal sperm (19.7%) compared to mice fed diets supplemented with 0.5 ppm Se (7.7%), with significant increases seen in head, tail, and multiple sperm abnormalities.

Kaur and Bansal (2009) demonstrated that in addition to an overall reduced fertility status associated with Se-deficient basal diet and the basal diet supplemented with 1.0 ppm sodium selenite (Se-excess), both deficient and excess groups of mice exhibit increased mRNA and protein expression of 70-kDa heat shock protein HSP70 whereas mRNA and protein expression patterns of HSP70-2 and MSJ-1 were decreased within the testis compared to their Se-adequate counterparts. These proteins are HSP70, the inducible heat shock protein, HSP70-2, the constitutive heat shock protein, and MSJ-1 is the chaperone protein for HSP70-2 required for its activity. These heat shock proteins can affect downstream factors that have a crucial role in differentiation of germ cells and the completion of spermatogenesis.

The exact function of HSP remains elusive but one role has been identified; HSP appear to protect organisms from adverse environmental conditions. One specific HSP, an abundantly expressed HSP70 plays an important role in thermotolerance (Li and Laszlo, 1985). Spinaci et al. (2005) demonstrated that HSP70 is present on boar sperm and goes through dynamic redistribution as the sperm undergoes capacitation and the acrosome reaction. In freshly ejaculated spermatozoa, HSP70 is present in a well-defined triangular-shaped area in the equatorial segment within the head of the spermatozoa. In sperm that have undergone the acrosome reaction, HSP70 translocates from inner to the outer leaflet of the sperm plasma membrane. This suggests that HSP may play an important role in porcine sperm-gamete

interaction. While Huang et al. (2000) reported low correlation coefficients between the level of HSP70 and semen quality traits, the semen quality traits tended to decline significantly in samples with lower levels of HSP70. Huang et al. (2002) found single nucleotide polymorphisms at sites 232, 250, and 345 in the 5'-flanking region of HSP70.2 are associated with semen quality of boars during the hot season that could be used as a tool for genetic screening. Kaushal and Bansal (2009) were the first to associate these HSP with oxidative stress and Se status, so more research needs to be done to find out whether these are Se-responsive elements in the pig and whether these could also be used as a possible genetic screening tool.

Concentrations of Se within plasma, serum, or whole blood are related to Se intake and are positively correlated with each other, but can vary with the form of Se fed (Ullrey, 1987). When defining blood Se status across livestock species: less than 50 ppb is considered deficient, 50 to 80 ppb is marginal, greater than 80 ppb is normal, and greater than 1 ppm is toxic (Koller, 1981). Based on blood Se levels, the boars used within this current investigation, including the unsupplemented control boars, had normal blood Se levels. As a consequence, it was not expected to observe semen characteristics as markedly affected as when Se is depleted. .

Bartle et al. (1980) did not find a difference in postcollection or post-thaw sperm characteristics of mature Holstein bulls injected with 0.055 ppm Se (as sodium selenite) at the start of the experiment followed by injections containing 0.11, 0.22, and 0.44 ppm Se at an increment of 10, 16, and 22 wk after the first injection, respectively. However, only three bulls were injected with Se and two bulls received the sham polysorbate injection. Segerson and Johnson (1981) injected 12 yearling Angus bulls with 50 mg of sodium selenite and 680 IU vitamin E initially and then repeated injections of 30 mg Se and 408 IU vitamin E every 21 days for a period of approximately 150 days; a second group of 12 yearling bulls was administered a

vehicle control on the same timeline. After 150 d, the bulls were electroejaculated, at which time semen was processed and frozen for subsequent examination, and the bulls were harvested for tissue collection. Semen characteristics of the fresh ejaculates prior to freezing were not recorded. Post-thaw semen viability and gross morphology characteristics were not different among treatments. There was also no difference in testis or epididymal weight or sperm concentration within the testis or epididymis.

Dimitrov et al. (2007) demonstrated a protective effect of supplementing turkey tom commercial diets with 0.3 ppm Sel-Plex on spermatozoa motility after 6 h of semen storage (10 to 15˚C water bath) as compared to a control diet that was only supplemented with 0.1 ppm sodium selenite. Motility of semen from Sel-Plex toms only decreased 4 relative percent, which was significantly different from the decrease seen in the semen from control toms which decreased 8.7 relative percent. Improvements in semen integrity in the Sel-Plex toms were also associated with improved in vivo fertility rates (90.5%) of stored spermatozoa compared to the control toms fed sodium selenite (88%). No mention was made whether the differences seen with in vivo fertility rates in this study were statistically significant. It is also important to note that the amount of Se supplemented to the two treatment groups was not an equivalent amount, so there could be a confounding factor of Se level in addition to Se source. No negative control animals were included.

Several studies have been conducted demonstrating positive effects of Se supplementation on reproductive characteristics in breeding boars (Segerson et al., 1981; Marin-Guzman et al., 1997, 2000a,b; Jacyno et al., 2002; Kolodziej and Jacyno, 2005). For example, Segerson et al. (1981) gave boars s.c. injections of sodium selenite (0.33 mg/kg BW) at 14-d

intervals, and reported increases in the number of spermatozoa per ejaculate but no effects on the percentages of viable or morphologically normal spermatozoa.

Marin-Guzman et al. (1997) were the first to report effects of supplemental selenium on boar fertility. Four experimental diets varying in Se (0 or 0.5 ppm sodium selenite) and vitamin E (0 or 220 IU/kg diet) were fed from weaning to sexual maturity. Semen was collected three times each week for 16-wk when the boars reached 9 mo of age. In general, both dietary Se and vitamin E enhanced boar semen quality, but the positive effects of added Se on semen characteristics were more pronounced than were the effects of added vitamin E. While there were no effects of treatment on semen volume, sperm concentration or total spermatozoa, sperm motility remained relatively constant in boars fed the Se-fortified diet compared to the unfortified diet. The percentage of normal spermatozoa declined in all treatment groups over the 16-wk period, but this decrease was least pronounced in boars receiving Se supplementation. After 8 wk, sperm from Se-fortified boars had the lowest incidence of spermatozoa with cytoplasmic droplets, whereas the sperm from unfortified boars had the highest incidence of cytoplasmic droplets. Sperm from Se-fortified boars also exhibited the least pronounced increase in bent and shoehook tails compared to those from unfortified boars. There were no differences in cytoplasmic droplet or bent and shoehook tails due to Vitamin E supplementation. Ejaculates were extended and semen used to inseminate gilts 12 and 24 h after the onset of estrus. Once bred, the gilts were sacrificed 5 to 7 d later. Boars supplemented with Se had increased fertilization rates (98.5% vs. 73.4%) and number of accessory spermatozoa penetrating the zona pellucida (59.7 spermatozoa vs. 14.2 spermatozoa) compared to unsupplemented boars. Vitamin E did not affect fertilization rates or numbers of accessory sperm.

In other reports (Marin-Guzman et al., 2000a,b), histological examination of testicular tissue of boars revealed that supplementation with Se (as sodium selenite), but not vitamin E, increased the number of Sertoli cells, round spermatids, and secondary spermatocytes. Marin-Guzman et al. (2000a) suggested that Se has a role in establishing the number of Sertoli cells and boar spermatozoal reserves. Also, Se deficient boars produced spermatozoa with decreased ATP concentrations, and electron microscopy revealed that these cells had structural abnormalities to the tail midpiece, including altered mitochondrial shape and orientation and poor contact of the plasma membrane to the helical coil.

More recently, Kolodziej and Jacyno (2004) evaluated semen characteristics in boars fed diets containing either 0.2 ppm Se and 30 ppm vitamin E, or 0.5 ppm Se and 60 ppm vitamin E, beginning at 70 d of age. There was no effect of treatment on gel-free semen volume or spermatozoa motility. In contrast, the concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the higher concentration of Se and vitamin E. Boars fed the diet containing the higher concentration of Se and vitamin E had higher percentages of spermatozoa with normal acrosomes and that passed a hypo-osmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities.

Mahan and Kim (1996) suggested that inorganic sources of Se, such as sodium selenite, may not be as biologically effective as the Se indigenous in cereal grains, which is primarily in an organic form, selenomethionine. Thus, we hypothesized that due to greater biological activity, an organic source of Se may enhance reproductive characteristics in boars to a greater extent than an equal level of sodium selenite, an inorganic source of Se. The results of the semen collection challenge and the semen storage experiments support this hypothesis, although the effects of the organic source of Se were not dramatic. Enhanced reproductive characteristics in

boars fed organic sources of Se compared to inorganic sources are supported by two reports in the literature.

Jacyno et al. (2002) compared organic and inorganic Se supplementation on semen characteristics in boars fed during either the winter or summer. Ejaculate volume and the percentage of motile spermatozoa were similar for boars fed diets supplemented with either 0.2 ppm organic Se (selenized yeast) and 60 ppm vitamin E, or 0.2 ppm inorganic Se (sodium selenite) and 30 ppm vitamin E beginning at 70 d of age. In contrast, the concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the organic source of Se. Boars fed the diet containing organic Se had higher percentages of spermatozoa with normal acrosomes and that passed a hypo-osmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities. Beneficial effects of the organic Se source were most evident in boars fed during the winter, but Se source was confounded with vitamin E concentrations in this study.

In the semen collection challenge portion of the current study, semen was collected on five consecutive d. Semen volume, sperm concentration, and the total number of sperm cells in ejaculates decreased with time, but these response variables were not affected by supplementation of diets with 0.3 ppm Se from inorganic or organic sources. However, characteristics of sperm motility also decreased with time, and the negative effects of day of semen collection on the percentages of progressively motile and rapidly moving spermatozoa, and measures of sperm velocity were least pronounced in boars fed diets supplemented with Sel-Plex.

Pruneda et al. (2005) found that stressing boars, by twice daily semen collections over a 4-d period alters resorption and secretion of the epididymal fluid, which results in defective

sperm maturation and development of sperm motility. Numbers of spermatozoa with proximal, intermediate, and distal cytoplasmic droplets were greater in ejaculates of stressed boars compared to control boars that had semen collected every two d. Sperm with abnormal head shape, size, and number and abnormal tail shape and numbers were greater in stressed boars compared to control boars.

Kumaresan et al. (2009) assessed boar sperm function of four ejaculates from each of four Hampshire boars during liquid semen preservation using BTS at 18˚C over 0, 24, 48, 72, and 96-h. Sperm motility, live sperm percentages, and percent of intact acrosomes all decreased over storage time. Lipid peroxidation levels, measured by malonaldehyde (MDA) levels, were low in sperm and seminal plasma at 0 h, but increased gradually over the 96-h period. The highest negative correlation was observed between MDA levels and sperm motility (r = -0.97), live sperm percent (r = -0.97), acrosomal integrity (r = -0.97), and hypo-osmotic sperm swelling test (HOSST) positive sperm percentage (r = -0.98). Percentages of HOSST positive sperm was strongly positively correlated with intact acrosome percentage (r = 0.98). Before preservation, 14.85 ± 4.66% of sperm cells showed low mitochondrial membrane potential, whereas this percentage increased to 32.00 ± 6.25% after 96-h of storage. The apoptotic sperm population was 8.33 ± 2.31% in fresh semen compared to 25.19 ± 4.25% at 96-h of preservation. This data indicates that liquid preservation at 18˚C induces lipid peroxidation of boar semen, decreases mitochondrial membrane potential, and decreases plasma membrane permeability, and could explain the impaired fertility seen with semen storage.

Guthrie et al. (2008) used flow cytometry assays to measure ROS formation, membrane lipid peroxidation, mitochondrial inner transmembrane potential ($\Delta\Psi_m$) during liquid storage and freeze-thawing boar semen for 120 min. Basal ROS formation and membrane lipid peroxidation

are minor in both fresh and stored boar semen, which the authors suggested to mean the endogenous ROS defense system in boar semen is either very efficient or virtually unchallenged. This would mean that there is either a low level of mitochondrial $\bullet O_2$ formation or sufficient enzymatic activity to neutralize $\bullet O_2$ and H_2O_2. Sperm in fresh, liquid-stored (17˚C in BTS) and frozen-thawed semen were equally susceptible to ROS generators including xanthine/xanthine oxidase, $FeSO_4$/ascorbate, and H_2O_2. Menadione, which is a direct inhibitor of the electron transport chain, inhibited sperm motility within 60 min and decreased $\Delta\Psi_m$ and sperm ATP content, while H_2O_2 led to the same decreases in sperm motility, but did not affect $\Delta\Psi_m$ or sperm ATP content. The authors suggested that instead of disrupting mitochondrial function, H_2O_2 instead exerted a negative effect directly on the axoneme to disrupt ATP utilization, or to interfere with the contractile mechanism within the axoneme itself. Measurements made during the semen storage experiment can be compared to sperm motility measurements for semen stored in BTS and Androhep Lite reported in a previously published paper by our laboratory (Estienne et al., 2007). Unlike the previously published paper, motility characteristics were not measured on day 1, but motility characteristics from d 3, 5, and 7 during storage at 18˚C can still be compared. One important thing to mention is while the same boars and the same diets were not used within these experiments, some of the variation seen in boars with different genetic backgrounds is reduced as we are comparing crossbred (Yorkshire x Landrace) boars. We also used the same laboratory equipment even though the person operating the equipment was different. In the semen storage experiment data presented herein, there were no effects due to extender x day or treatment x extender x day, so data were pooled between extenders. This disagrees with the previously published report, however that paper compared the motility characteristics of semen samples stored in nine different extenders, only two of which were used

in this current paper. In that paper, sperm velocity measures (VSL) were greater for Androhep Lite semen samples compared to BTS on d 1 and 3, while sperm path measurements such as straightness and linearity were greater for sperm stored in Androhep Lite compared to BTS at d 3, 5, and 7, which can be indicative of the fact that Androhep is a longer-term extender, compared to BTS, which is considered a short-term extender.

In the semen storage experiment, pH increased during storage in semen collected from control boars and boars fed a diet supplemented with selenite compared to Sel-Plex supplemented boars. Whether the changes in pH during storage are the cause of, or are somehow related to, the differences in motility characteristics remains to be determined. However, in a study comparing five different commercial extenders for boar semen, pH of stored semen increased from day 1 to day 7, and pH was negatively correlated with sperm motility. The changes in pH could not be attributed to factors such as bacterial contamination or the percentage of dead spermatozoa (Vyt et al., 2004b). A subsequent report (Vyt et al., 2007) demonstrated that these increases in pH seen with stored semen was due to loss of CO_2 from the extender into the airspace within the tube or AI bottle and was increased with increasing air volumes within the semen storage vessel. Adding CO_2 to the atmosphere resulted in minimal pH changes within the stored semen. While outside the scope of this investigation, pH could be a response variable that the swine industry wants to pay more attention to and research further in the future.

In commercial boar studs, sperm motility assessment is a technique used routinely for semen evaluation (Shipley, 1999). It is acknowledged, however, that like any on-farm laboratory test, assessment of sperm motility does not provide a completely accurate or quantitative measure of semen fertility per se. Visual motility assessment tends to be highly subjective and in many boar studs is being replaced with more objective detailed and repeatable assessments using

computerized systems (Vyt et al., 2004b). Moreover, Holt et al. (1997) demonstrated that up to 24% of the variance in litter size due to boars on commercial swine farms could be explained by differences in sperm motion characteristics determined using CASA technology. Boar spermatozoa that exhibited increased VSL and track linearity were associated with larger litter sizes.

None of these tests, however, estimate actual sperm function such as binding to and penetrating the zona pellucida (Gadea, 2005). Oocyte penetration rates are more accurate measures of boar fertilization capacity and interactions between the spermatozoa and the oocyte plasma membrane could perhaps explain variability of fertilization capacity seen within fertile boars (Gadea, 2005). Instead of using IVF to predict fertility of all boars within a commercial boar stud, Ruiz-Sanchez et al. (2006) suggested that IVF could be more useful to screen boars of questionable fertility.

As reviewed by Gil et al. (2008), there are a number of different variables that can also affect in vitro penetration and polyspermy rates. Large variations have been reported among boars in fertilization rates with both fresh and frozen boar spermatozoa. Individuals within a breed, different ejaculates collected from the same boar, and even fractions within a given ejaculate from one boar can vary considerably. Eliminating some of this inter- and intra-boar variability would lead to more standardized porcine IVF procedures. In earlier studies fresh spermatozoa was the main source of spermatozoa for IVF because of the problems involved in cryopreservation of boar spermatozoa, but the improvement of those protocols improved post-thaw sperm viability sufficiently enough to eliminate some variation you would have with fresh spermatozoa otherwise. The variability of sperm characteristics such as motility, viability, morphology, and acrosome status can sometime correlate well with in vitro fertility in some

boars, and yet correlate poorly with others. This means that one cannot accurately estimate the relationship between sperm freezability or storage characteristics and in vitro fertility based on a single ejaculate. Sperm concentration, or sperm:oocyte ratios is one of the most important variables in IVF systems as it is highly correlated with polyspermy incidence. Co-incubation time is another variable that can inflate both penetration and polyspermy rates. The authors specify that most current IVF systems use a 5- to 6-h sperm-oocyte co-incubation time, compared to 12- to 18-h used in the original porcine IVF systems. Culture medium components can also greatly impact in vitro fertilization rates.

Xu et al. (1998) demonstrated a number of problems that can occur with porcine IVF systems. In addition to large variations between boars used for IVF, semen dilution rates have significant effects on fertilization rates. They discovered a quadratic relationship between sperm:oocyte ratios and oocyte penetration rates, MPN formation rates, and potential embryo production rates. As sperm:oocyte ratios increase, the number of sperm attached to the plasma membrane increases linearly. In their experimentation potential embryo production rates were the most important predictor of sperm quality, because the variable took into account penetration rate, monospermy rate, and MPN formation rate.

However, one big advantage of using IVF not mentioned in the literature is one of labor, cost, and space requirements. Marin-Guzman et al. (1997) *in vivo* fertilization experiment required the use of approximately 3 to 4 mature gilts inseminated per boar that were later sacrificed 5 to 7-d after AI to recover the reproductive tracts. For the 18 boars that were used for our IVF experimentation, we would have needed at least 120 gilts to inseminate assuming a 90% conception rate with each semen sample (stored for 1 d and 8 d, respectively). Xu et al. (1998) used breeding data from 444 weaned sows bred to six boars used in their IVF procedures to

correlate *in vivo* fertility with *in vitro* IVF estimates and standard laboratory semen quality measurements. Using that many sows for the purposes of extending our research would require the cooperativity of multiple commercial sow farms, the logistics of which seem monumental. Funding the purchase of 120 gilts, operating costs such as electricity, feed and veterinary supplies and labor to take care of the gilts would be high While the IVF experimentation was not without cost, especially with purchasing porcine oocytes, in the long run it probably saved some money.

In the current investigation we used crossbred Yorkshire x Landrace boars that are similar to what one would encounter in the U.S. commercial swine industry. Heterosis could eliminate some of the variation that one might encounter if they were comparing in vitro fertilizing capacities of a terminal-line Hampshire or Duroc boars compared to that seen in a maternal Yorkshire or Landrace boar. As such, this could also eliminate a little more of the inter-boar variation seen with IVF procedures as reported by (Xu et al., 1998; Gil et al., 2008).

It can be difficult to compare fertilization rates between those fertilization rates on d 1 after semen collection reported herein with those reported in the literature (Xu et al., 1998; Whitaker and Knight, 2004; Gil et al., 2008) considering nobody used the exact methodology or the same breeds of boar, semen extenders, semen dilution rates, or sperm:oocyte number as the methods developed within our laboratory (Whitaker and Knight, 2004) with the exception of using liquid semen stored at 18˚C. A recent review by Gil et al. (2010) states that the efficiency of IVF (percentage of monospermic oocytes from total inseminated oocytes) is approximately 30 to 50% in most laboratories. In this case, some of the fertilization rates reported herein are higher than 30 to 50 % on day 1 of semen storage, but the values on d 8 are closer to those values. Differences could be attributed to a myriad of factors previously discussed in differences

in IVF methodology. A paucity of information exists in the literature on the effect of semen stored at 18˚C for 8-d to know how well that data corresponds to what is in the literature.

In vitro penetration rates published by Xu et al. (1998) vary from 66.1% for the highest sperm:oocyte rate (3125:1), 75.3% for the intermediate sperm:oocyte rate (12500:1), and 82.8% for the lowest sperm:oocyte dilution rate (50000:1). Oocyte penetration rates were found to be proportional with polyspermy rates and inversely proportional with monospermy rates. Thus, the highest semen dilution rate exhibited 58.9% polyspermy and 41.1% monospermy rate, the intermediate dilution exhibited 68.2% polyspermy and 31.8% monospermy, and the lowest dilution exhibited 78.1% polyspermy and 21.9% monospermy. These dilution rates are less dilute than the procedures developed by our laboratory (approximately 1666 sperm:oocyte); One could speculate that our results would show decreased oocyte penetration while increasing percentages of monospermic penetration and decreasing polyspermic penetration based on semen dilution principles alone.

Consistent with previous work from our laboratory, however, there was no difference between extenders with regard to maintenance of motility characteristics. Throughout 10-d semen storage, the values of the various sperm motility measurements were in general greatest for the Sel-Plex supplemented boars compared to unsupplemented boars or selenite supplemented boars. These results suggest that sperm cells collected from boars fed diets supplemented with an organic source of Se remain viable for a longer period of time during liquid storage. In the IVF experiment, supplementation of boar diets with organic selenium improved IVF capability 1-d after semen collection and tended to improve fertilizing capability of spermatozoa 8 d after semen collection.

The precise mechanism by which negative effects of day during a semen collection challenge are minimized, motility characteristics during liquid semen storage were maintained to a greater extent, and increased fertilization rates seen during liquid semen storage when boars are fed organic Se remains to be elucidated and warrants further scrutiny.

Table 4-1. Boar Diet Formulations

	Dietary Treatment[a]		
Ingredient	**Control**	**Selenite**	**Sel-Plex**
Corn	83.57	83.57	83.57
Soybean meal	13.00	13.00	13.00
Dicalcium phosphate	1.90	1.90	1.90
Calcium carbonate	0.85	0.85	0.85
Salt	0.35`	0.35	0.35
Vitamin premix[b]	0.05	0.05	0.05
Choline chloride (60% choline)	0.15	0.15	0.15
Trace mineral premix[c]	0.08	0.08	0.08
Copper sulfate	0.10	0.10	0.10
Filler[d]	0.05	--	--
Sodium Selenite premix[e]	--	0.05	--
Selenized Yeast premix[f]	--	--	0.05

[a] Diets were prepared by first mixing a basal diet that included ingredients common to each test diet. Individual experimental diets were then prepared by thoroughly mixing the appropriate test ingredient (filler, sodium selenite, or selenized yeast) with the appropriate quantity of basal diet. Experimental diets contained 0.034 ppm, 0.316 ppm, and 0.320 ppm Se, respectively, for diets containing no supplemental Se, sodium selenite, and selenized yeast.

[b] Provided per kg of complete diet: 11,023 IU vitamin A, 1,102 IU cholecalciferol, 66 IU vitamin E, 2 mg vitamin K as menadione, 30 µg vitamin B_{12}, 30 mg niacin, 28 mg pantothenic acid, 8 mg riboflavin, 0.358 mg biotin, 2 mg folic acid, 5 mg pyridoxine, and 2 mg thiamin

[c] Provided per kg of complete diet: 107 mg iron sulfate, 133 mg zinc oxide, 53 mg manganese oxide, 16 mg copper sulfate, and 1.36 mg iodine.

[d] Filler consists of a mixture of equal portions of ground limestone and ground corn to fill 0.05% of the basal diet

[e] Premium Selenium 270 (North American Nutrition Co., Inc., Lewisburg, OH) containing 600 ppm Se as sodium selenite

[f] Sel-Plex (Alltech Inc., Nicholasville, KY) containing 600 ppm Se as selenized yeast product

Table 4-2. Characteristics of semen from boars collected on five consecutive d and that were fed a control diet that contained no supplemental Se or diets supplemented with 0.3 ppm Se from organic (Sel-Plex) or inorganic sources (sodium selenite). Values are LS Means ± SE.

	Dietary Treatment				*P*- values		
Item	Control	Sodium Selenite	Sel-Plex	SE	Treatment	Day	Treatment x Day
Number of boars	10	10	10	---	---	---	---
Volume, mL	167.4	147.9	144.2	10.8	0.28	<0.01	0.29
Gel, g	40.0	36.2	26.4	5.5	0.22	<0.01	0.72
Sperm concentration, million/mL	225.1	183.8	242.9	27.6	0.31	<0.01	0.13
Total sperm cells, billions	36.6	27.7	35.0	3.6	0.19	<0.01	0.81
AI doses[1]	12.0	8.8	11.0	1.2	0.18	<0.01	0.50
Characteristics of spermatozoa as determined by computer-assisted sperm analysis system							
Motile, %	97.1	93.0	96.6	2.2	0.36	0.05	0.23
Progressively motile, %	67.6	60.7	69.7	4.1	0.29	0.01	0.02
VAP[2], µm/sec	104.7	103.5	107.4	3.3	0.68	0.06	0.05
VSL[3], µm/sec	75.3	73.4	79.3	3.3	0.43	0.03	0.05
VCL[4], µm/sec	195.5	191.2	197.7	6.5	0.77	0.09	0.08
ALH[5], µm	8.0	7.7	7.9	0.2	0.48	0.11	0.15
BCF[6], Hz	35.6	35.9	35.6	0.7	0.93	0.04	0.44
STR[7], %	69.8	69.1	71.3	1.4	0.49	0.16	0.90
LIN[8], %	38.9	39.4	40.1	1.3	0.81	0.78	0.33
Elong[9], %	48.0	47.8	46.9	0.4	0.17	0.40	0.55
Area[10], µm sq	11.7	11.5	12.2	0.2	0.06	<0.01	0.02
Rapid, %	80.3	74.5	81.1	4.0	0.45	0.07	0.03
Medium, %	7.2	6.6	6.4	0.7	0.69	0.04	0.40
Slow, %	9.6	12.0	9.3	2.1	0.60	0.03	0.44
Static, %	2.9	7.0	3.4	2.2	0.36	0.05	0.23
Sperm morphology							
Normal, %	89.7	85.3	89.9	3.0	0.48	0.63	0.90
Abnormal head, %	0.5[a]	0.1[b]	0.4[a,b]	0.1	0.02	0.19	0.91
Abnormal tail, %	2.2	1.9	2.9	1.0	0.74	0.08	0.82
Detached head, %	1.6	2.5	0.7	1.0	0.34	0.06	0.91
Bent tail, %	0.4	0.3	0.7	0.2	0.42	0.44	0.85
Proximal droplet, %	2.0	7.2	1.7	2.0	0.12	0.10	0.74
Distal droplet, %	3.5	2.8	3.8	0.8	0.59	0.58	0.39
Other abnormalities, %	0.02	0.00	0.02	0.01	0.61	0.63	0.36

[1]Three billion sperm cells/AI dose.
[2]VAP= Path velocity of the smoothed cell path.
[3]VSL= Average velocity measured in a straight line from the beginning to the end of track.
[4]VCL= Average velocity measured over the actual point to point track followed by the cell.
[5]ALH= Amplitude of lateral head displacement corresponding to the mean width of the head oscillation as the sperm swam.
[6]BCF= Frequency with which the sperm track crossed the sperm path (i.e., frequency of sperm head crossing the sperm average path in either direction).
[7]STR= Average value of the ratio VSL/VAP; Measured the departure of the cell path from a straight line.
[8]LIN= Average value of the ratio VSL/VCL; Measured the departure of the cell track from a straight line.
[9]Elong= Average value of the ratio of minor to major axis of all sperm heads.
[10]Area= Average size of all sperm heads.
[a,b]Within a row, means with different superscripts differ (P <0.06).

Table 4-3. Characteristics of extended semen from boars fed a control diet that contained no supplemental Se or diets supplemented with 0.3 ppm Se from organic (Sel-Plex) or inorganic sources (sodium selenite) and was stored at 18˚C for 10-d[1].

Item	Dietary Treatment			SE	*P*-value		
	Control	Selenite	Sel-Plex		Treatment	Day	Treatment x Day
Number of boars	10	10	10	---	---	---	---
pH	6.91	6.90	6.85	0.05	0.72	< 0.001	< 0.001
Characteristics of spermatozoa as determined by computer-assisted sperm analysis system							
Motile, %	89.72	80.37	89.64	4.53	0.26	< 0.001	0.009
Progressively motile, %	41.13	41.38	47.40	5.87	0.70	< 0.001	0.26
VAP[2], µm/sec	69.23	67.96	77.64	6.06	0.48	< 0.001	0.06
VSL[3], µm/sec	49.48	48.55	55.57	4.46	0.49	< 0.001	0.31
VCL[4], µm/sec	131.72	133.50	148.39	11.19	0.52	0.49	0.97
ALH[5], µm	7.54	6.75	7.85	0.32	0.06	0.25	0.02
BCF[6], Hz	34.49	34.20	33.74	0.50	0.58	< 0.001	0.04
STR[7], %	69.49	68.11	69.07	1.17	0.69	< 0.001	0.01
LIN[8], %	39.36	38.60	38.79	0.91	0.83	< 0.001	0.27
Elong[9], %	46.66	43.8	45.33	0.83	0.07	< 0.001	0.12
Area[10], µm sq	14.96	14.67	15.62	0.53	0.44	< 0.001	0.04
Rapid, %	47.53	47.29	54.57	6.63	0.68	< 0.001	0.21
Medium, %	24.20	18.39	20.72	1.96	0.12	< 0.001	0.88
Slow, %	17.99	14.72	14.37	2.37	0.50	< 0.001	0.11
Static, %	10.28	19.64	10.31	4.53	0.26	< 0.001	0.009
Sperm morphology							
Normal, %	85.53	84.93	85.49	2.07	0.97	0.54	0.59
Abnormal head, %	1.12	1.11	0.96	0.26	0.89	0.51	0.61
Abnormal tail, %	1.60	1.23	1.66	0.53	0.83	0.35	0.24
Detached head, %	7.52	8.47	6.52	1.13	0.49	0.87	0.88
Bent tail, %	1.21	1.62	1.55	0.38	0.72	0.05	0.74
Proximal droplet, %	0.67	0.55	0.57	0.24	0.94	0.89	0.69
Distal droplet, %	2.37	2.10	3.26	0.49	0.23	0.78	0.84
Other abnormalities, %	0.00	0.00	0.00	0.00	1.00	1.00	1.00

[1]Semen was extended in Androhep-Lite (Minitube of America, Inc.) and Beltsville Thawing Solution (Minitube of America, Inc.) but because there was no effect of day x extender or treatment x day x extender, data was pooled between semen extenders. Data are LS Means ± SE.
[2]VAP= Path velocity of the smoothed cell path.
[3]VSL= Average velocity measured in a straight line from the beginning to the end of track.
[4]VCL= Average velocity measured over the actual point to point track followed by the cell.
[5]ALH= Amplitude of lateral head displacement corresponding to the mean width of the head oscillation as the sperm swam.
[6]BCF= Frequency with which the sperm track crossed the sperm path (i.e., frequency of sperm head crossing the sperm average path in either direction).
[7]STR= Average value of the ratio VSL/VAP; Measured the departure of the cell path from a straight line.
[8]LIN= Average value of the ratio VSL/VCL; Measured the departure of the cell track from a straight line.
[9]Elong= Average value of the ratio of minor to major axis of all sperm heads.
[10]Area= Average size of all sperm heads.

Table 4-4. Effects of dietary supplementation with an organic (Sel-Plex) or inorganic (sodium selenite) source of selenium on in-vitro fertilization of stored boar spermatozoa.[1]

					P-values		
Item:	Control	Sodium Selenite	Sel-Plex	SE	Treatment	Day	Treatment x Day
Number of boars	6	6	6	---	---	---	---
Oocyte number							
Unfertilized	36.2	37.8	26.4	3.4	0.20	0.03	0.85
Monospermic	45.1	43.1	49.1	2.9	0.32	0.15	0.10
Polyspermic	11.3	12.5	13.9	1.9	0.58	0.06	0.42
MPN[2]	52.6	55.1	60.3	4.9	0.48	0.13	0.15
Total oocytes	92.6	87.4	89.4	4.5	0.69	0.49	0.60
Percentage of total oocytes fertilized							
Fertilization rate	60.9	63.7	70.7	3.4	0.10	0.03	0.59
Monospermy rate	48.9	49.6	55.1	2.7	0.19	0.07	0.57
Polyspermy rate	11.9	14.1	15.6	1.9	0.34	< 0.01	0.91
MPN rate	56.4	62.7	67.8	4.3	0.16	0.04	0.67

[1]Data was pooled across length of storage (IVF conducted 2 and 8 d into storage at 18˚C, with semen collection being d 0) because the interaction of day x treatment was not ($P \geq 0.10$) significant. Values are LSMeans ± SE.
[2]Oocytes with male pronuclear (MPN) formation.

Figure 4-1. Percentage of sperm cells displaying progressive motility in semen collected from boars (n = 10 per treatment) over five consecutive d fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LS means $\pm$ SE. A treatment x day interaction ($P = 0.02$) was detected. At d 5, the percentage of sperm cells displaying progressive motility was greater ($P < 0.02$; *) for boars fed Sel-Plex compared with the other two groups.

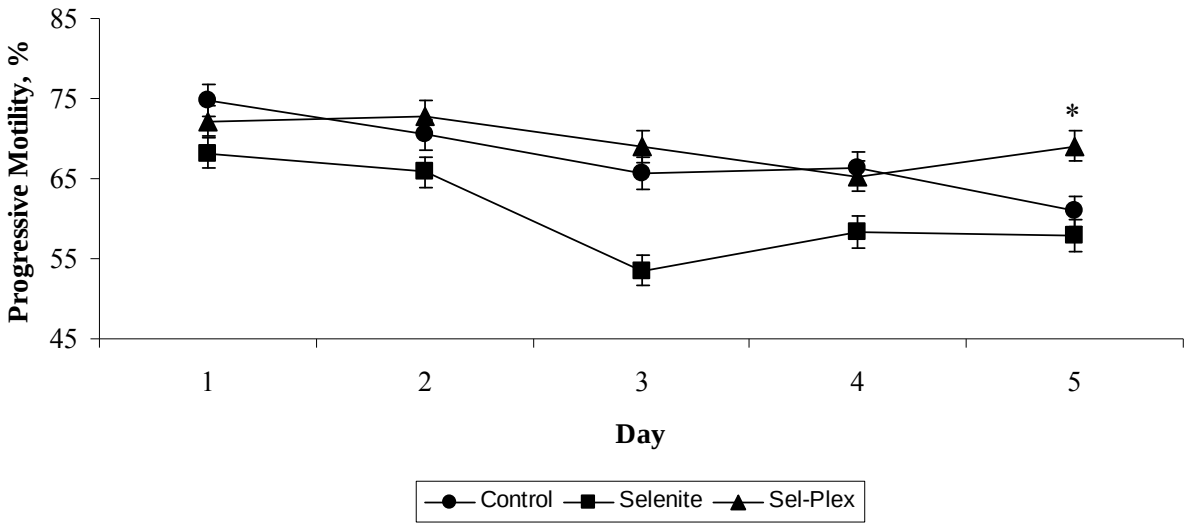

85
75
65
55
45
Progressive Motility, %
*
1
2
3
4
5
Day
Control
Selenite
Sel-Plex

Figure 4-2. Percentage of sperm cells displaying rapid motility in semen collected from boars (n = 10 per treatment) over five consecutive d fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LS means $\pm$ SE. A treatment by day interaction ($P = 0.03$) was detected. At d 5, the percentage of sperm cells displaying rapid motility was greater ($P < 0.03$; *) for boars fed Sel-Plex compared with the other two groups.

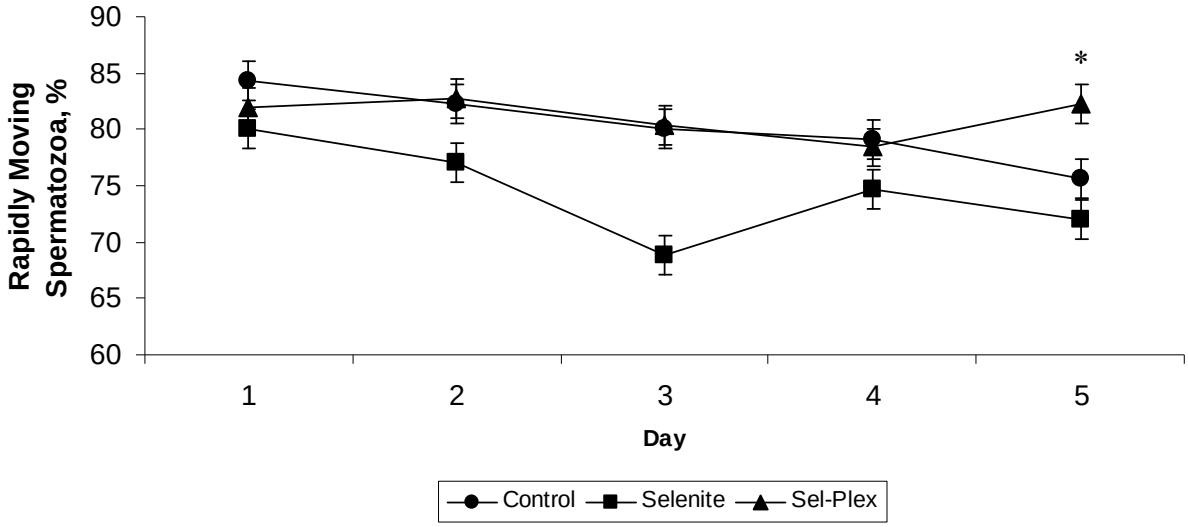

Rapidly Moving Spermatozoa, %
90
85
80
75
70
65
60
1
2
3
4
5
Day
*
Control
Selenite
Sel-Plex

Figure 4-3. Path velocity (VAP; μm/s) for spermatozoa in semen collected from boars (n = 10 per treatment) over five consecutive d fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LS means $\pm$ SE. A treatment by day interaction ($P = 0.05$) was detected. At d 3, the VAP of sperm cells was greatest ($P < 0.01$; *) for boars fed Sel-Plex and control diets compared to boars fed diets supplemented with sodium selenite.

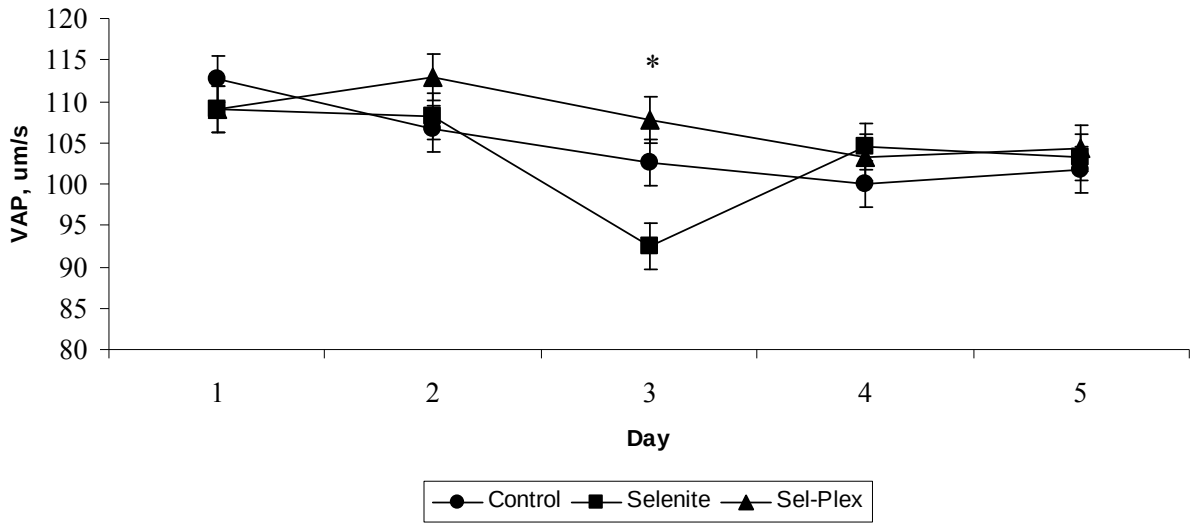

120
115
110
105
100
95
90
85
80
VAP, um/s
*
1
2
3
4
5
Day
Control
Selenite
Sel-Plex

Figure 4-4. Progressive velocity (VSL; μm/s) for spermatozoa in semen collected from boars (n = 10 per treatment) on five consecutive d fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LS means ± SE. A treatment by day interaction ($P = 0.05$) was detected. At day two and three, VSL tended to be greater ($P < 0.06$; *) for boars fed Sel-Plex compared with the other two groups.

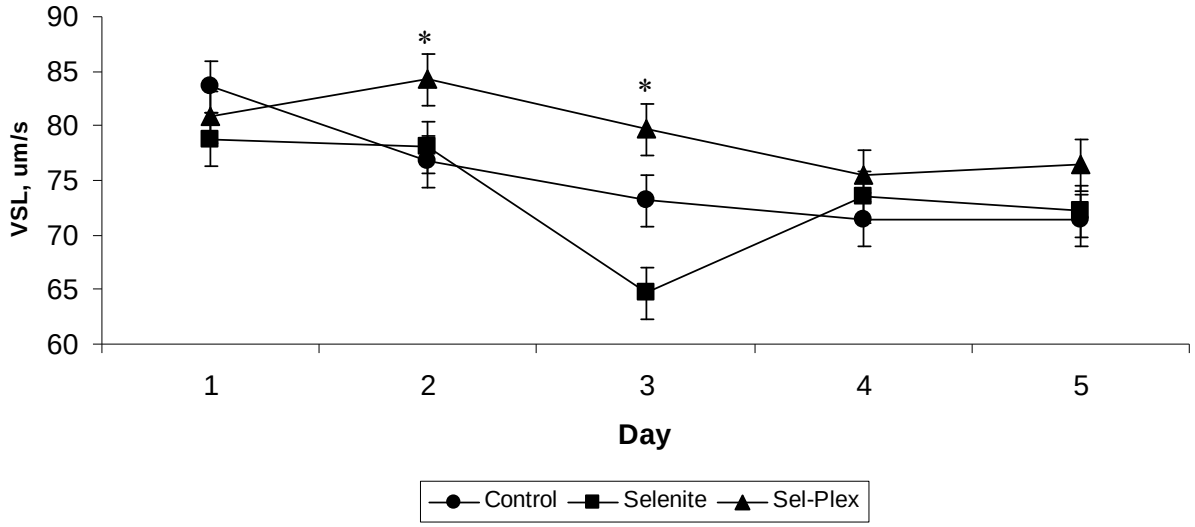

90
85
80
75
70
65
60
VSL, um/s
*
*
1
2
3
4
5
Day
Control
Selenite
Sel-Plex

Figure 4-5. Size of heads (μm^2) of spermatozoa in semen collected from boars (n = 10 per treatment) collected over five consecutive d fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources. Values are LS means $\pm$ SE. An effect of treatment x day ($P = 0.02$) was detected. On d one and two, the size of sperm heads was greater ($P < 0.02$; *) for boars fed Sel-Plex compared with the other two groups. On d three, the size of sperm heads was least ($P < 0.02$;) for boars fed selenite compared with the other two groups.

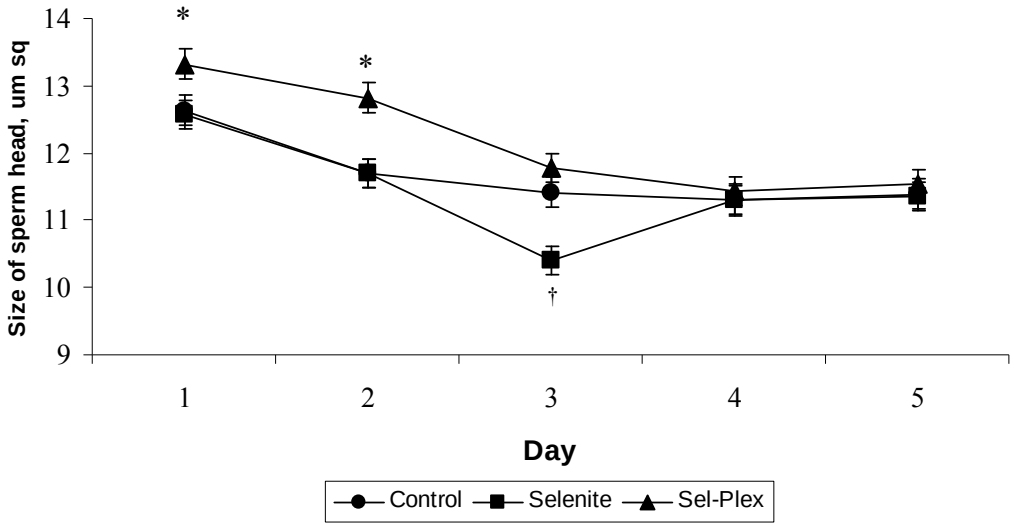

Size of sperm head, um sq
14
13
12
11
10
9
*
*
†
1
2
3
4
5
Day
Control
Selenite
Sel-Plex

Figure 4-6. The percentage of motile sperm cells in semen collected from boars (n = 10 per treatment) fed a control diet that contained no supplemental Se or diets supplemented with 0.3 ppm organic Se (Sel-Plex) or inorganic Se (sodium selenite) and stored at 18˚C for 10-d. Values are LS means $\pm$ SE. A treatment x day interaction (P = 0.009) was detected. On d 9, spermatozoa from Sel-Plex fed boars exhibited greater (P = 0.01; *) motility compared to spermatozoa from selenite-fed boars. On day 10, spermatozoa from control and Sel-Plex boars exhibited greater ($P < 0.01$; †) motility compared to spermatozoa from boars supplemented diets with sodium selenite.

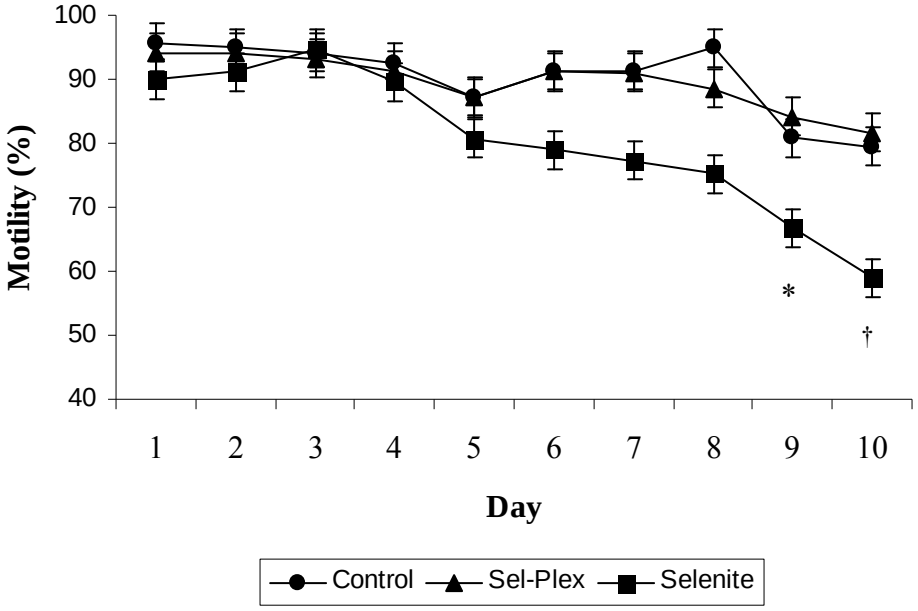

100
90
80
70
60
50
40
Motility (%)
1
2
3
4
5
6
7
8
9
10
Day
*
†
Control
Sel-Plex
Selenite

Figure 4-7. Percentage of static spermatozoa in semen collected from boars (n = 10 per treatment) fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources and stored at 18˚C for 10-d. Values are LS means ± SE. A treatment x day interaction ($P = 0.009$) was detected. On d 9, spermatozoa from selenite fed boars exhibited greater ($P = 0.01$; *) proportions of static spermatozoa compared to spermatozoa from boars supplemented with Sel-Plex. On day 10, spermatozoa from boars supplemented with sodium selenite exhibited greater ($P < 0.01$; †) proportions of static spermatozoa compared to spermatozoa from boars fed the control diet or those supplemented with Sel-Plex.

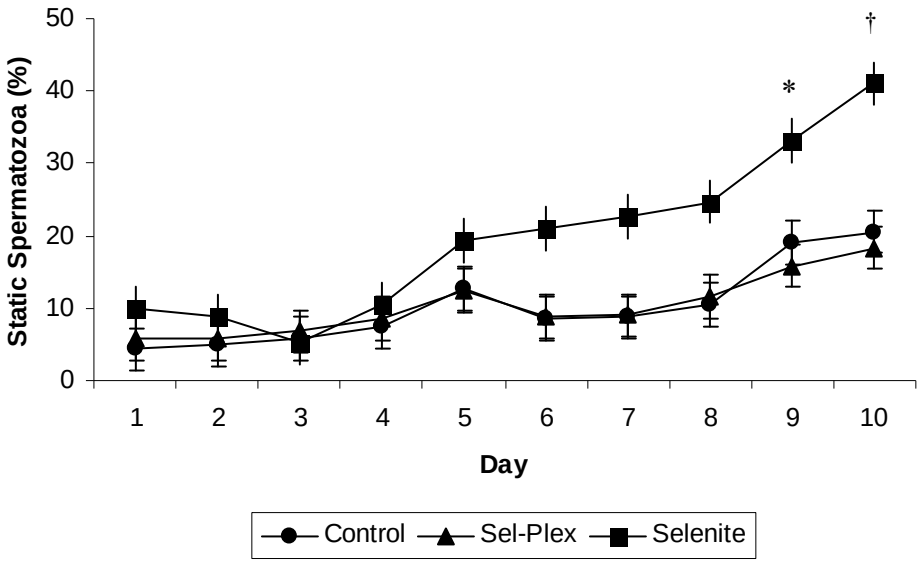

50
40
30
20
10
0
Static Spermatozoa (%)
1
2
3
4
5
6
7
8
9
10
Day
*
†
Control
Sel-Plex
Selenite

Figure 4-8. Amplitude of lateral head displacement (ALH, μm) for spermatozoa in semen collected from boars (n = 10 per treatment) fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources and stored at 18˚C for 10-d. Values are LS means ± SE. A treatment by day interaction ($P = 0.02$) was detected. On d 7 and 10, spermatozoa from boars fed the control diet and diet supplemented with Sel-Plex exhibited greater ($P < 0.05$; *) head displacement compared to spermatozoa from the selenite supplemented boars. On d 9, only spermatozoa from Sel-Plex supplemented boars exhibited greater ($P < 0.01$; †) ALH compared to spermatozoa from selenite supplemented boars.

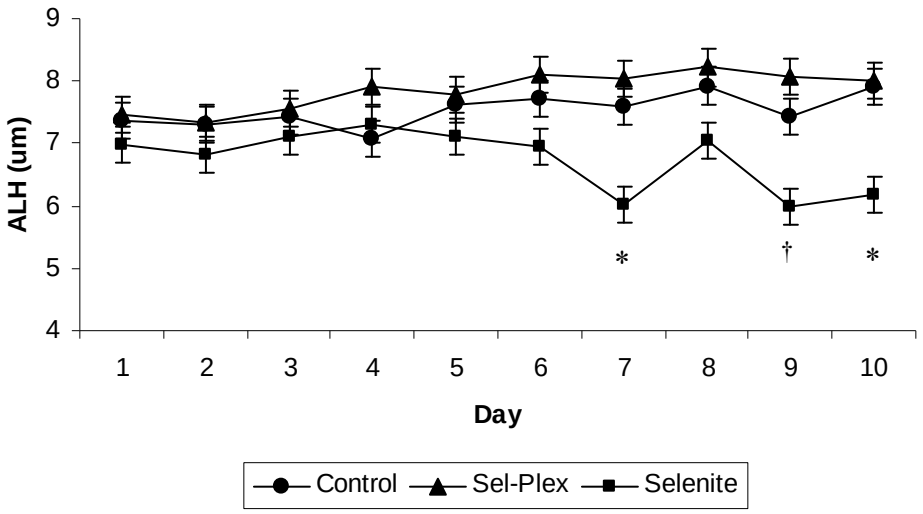

9
8
7
6
5
4
ALH (um)
1
2
3
4
5
6
7
8
9
10
Day
*
†
*
Control
Sel-Plex
Selenite

Figure 4-9. Straightness (STR, %) for spermatozoa in semen collected from boars (n = 10 per treatment) fed the control diet or diets supplemented with Se from organic (Sel-Plex) or inorganic (sodium selenite) sources and stored at 18˚C for 10-d. Straightness is defined as the average value of the ratio VSL/VAP; measured by the departure of the cell path from a straight line. Values are LS means $\pm$ SE. A treatment x day interaction ($P = 0.01$) was detected. On d 10, spermatozoa from boars fed the control diet and diet supplemented with Sel-Plex exhibited greater straightness ($P < 0.01$; *) compared to spermatozoa from boars supplemented with sodium selenite.

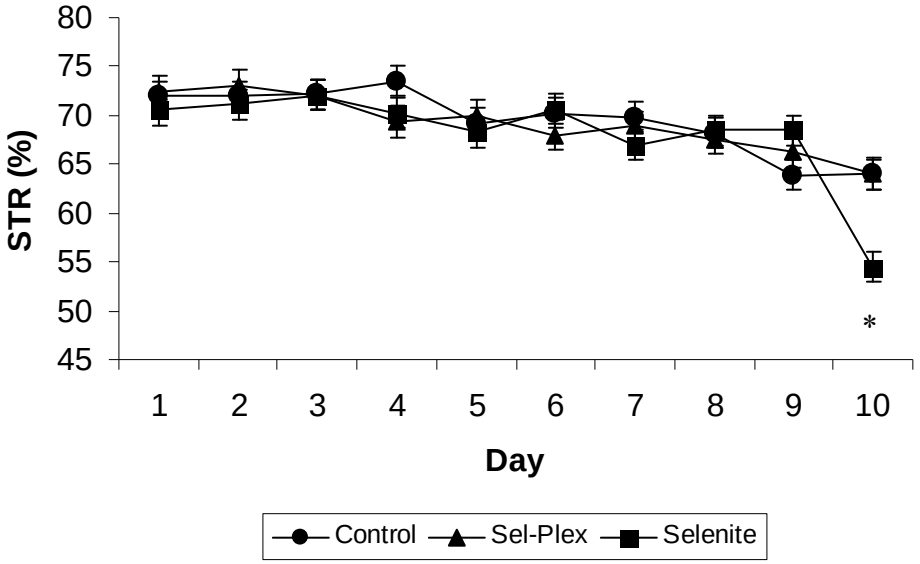
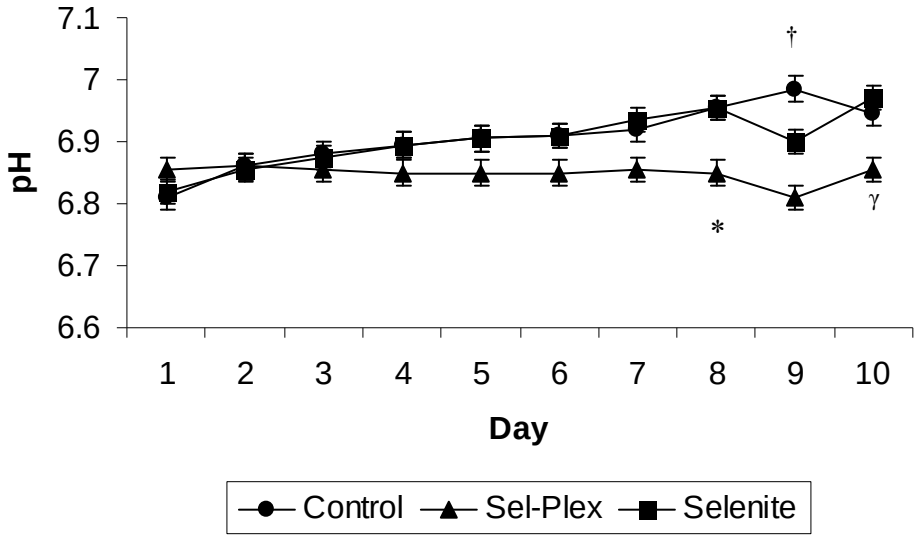

80
75
70
65
60
55
50
45
STR (%)
1
2
3
4
5
6
7
8
9
10
Day
*
Control
Sel-Plex
Selenite

Figure 4-10. The pH of semen collected from boars (n = 10 per treatment) fed the control diet or diets supplemented with organic (Sel-Plex) or inorganic (sodium selenite) sources and stored at 18˚C for 10-d. Values are LS means ± SE. A treatment x day interaction ($P < 0.01$) was detected. On d 8, there was a tendency for the extended semen of Sel-Plex supplemented boars to have a lower pH ($P = 0.08$; *) compared to the extended semen of the boars supplemented with sodium selenite (6.85 vs. 6.96, respectively; SE = 0.02). On d 9, extended semen from boars supplemented with Sel-Plex exhibited the lowest ($P = 0.003$; †) pH (6.81) compared to extended semen from boars fed the control diet, which had the highest pH (6.98); semen from boars supplemented with sodium selenite had an intermediate pH (6.90; SE = 0.02) that was not significantly different from the other treatment groups. On d 10, pH of extended semen from Sel-Plex supplemented boars was the lowest ($P < 0.02$) compared to semen from control and selenite-supplemented boars.


7.1
7
6.9
6.8
6.7
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pH
1
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Selenite

CHAPTER FIVE

SUMMARY and IMPLICATIONS

Since the discovery of Se as an essential trace mineral, and the discovery that Se is a component of glutathione peroxidase, the importance of Se in human and animal biochemistry and physiology has become unquestionable. Research is continuing to unravel new aspects of the molecular biology of Se- from its incorporation into proteins to its role in gene expression reproduction and cancer research. What I would like to do here is hone in on the role that selenium could play in swine nutrition and reproductive physiology. I will highlight data already presented within my dissertation and present some areas of research that I would like to pursue. There certainly are a lot more published reports on the effect of selenium deficiency and toxicosis and Se supplementation in the rodent model. By comparison, there are much fewer published reports investigating the impact that Se has in the pig.

The data presented herein helps bridge some of the substantial gaps that existed regarding the impact that organic Se supplementation has on boar growth and reproductive physiology. The research reported within this dissertation certainly does not answer all questions, in fact it leaves even more questions for potential research to answer in the coming years.

There are several experiments I would like to conduct. One series of experiments I would like to conduct would be to look at the Se requirement of the boar, conducting experiments similar to those that are included within this dissertation. In addition to examining the source of Se, the Se requirements established by the NRC were, by in large, developed for the sow and gilt, not the breeding boar. The treatments would include a basal diet with no additional Se, with three levels of sodium selenite and Sel-Plex (0.3 ppm, 0.5 ppm, and 1 ppm Se). The boars would have to be on their diets from weaning like these experiments were, as

Sertoli cell number is fixed prior to puberty. Once trained to allow for semen collection, I would like to conduct experiments such as those included in Chapter 4 of this dissertation. Since Virginia does have a summer heat and humidity problem, would temperature and/or humidity affect sperm parameters, and would dietary Se inclusion serve a protective function in the boar like the heat shock proteins might in some cases of seasonal infertility?

Kaushal and Bansal (2009) reported that in addition to an overall reduced fertility status caused by Se-deficient and Se-excess diets (basal diet supplemented with 1.0 ppm sodium selenite), there was increased mRNA and protein expression of HSP70, a heat shock protein. Differentially regulating HSP70s can affect downstream factors that have a crucial role in differentiation of germ cells and the completion of spermatogenesis. While HSP70 was poorly correlated with sperm motility, HSP70-2 appears to have more promise, at least with seasonal infertility. Five SNPs were associated with semen quality and seasonal infertility in the boar (Huang et al., 2002), but no additional research has been published. I would like to see whether boars that are deficient in Se or are supplemented with different forms of Se express different levels of HSP70-2 or its chaperone protein that controls the expression of HSP70-2.

Another possible route research could follow with the same animals would be continuing the same dietary treatments and looking into the impact of ROS, mitochondrial function, and their relation to sperm motility.

With an endless supply of funding and people to do lab work, we could also evaluate the boars for the effect of Se supplementation on freeze-thawing. This could be either more applied in focus, just looking at sperm motility measurements using CASA and morphology assessments, and even IVF capacity, or a more molecular in focus by conducting ROS, HSP, and/or GPx4 assays.

As discussed in Chapter 3 of this dissertation, supplementing boar diets with 0.3 ppm organic Se (Sel-Plex) improved G:F during the grow-finish phase compared to boars receiving an equivalent amount of inorganic Se (sodium selenite) or an unsupplemented basal diet. This was a finding that was not consistent with findings published in barrow and gilt studies (Mahan and Parrett, 1996; Mahan et al., 1999; Mateo et al. 2007). The dichotomy was not easily explained, but one could hypothesize that organic Se supplementation could be increasing testosterone secretion, which could lead directly or indirectly to improvements in feed efficiency. While some studies established that Se-deficient mice and rats exhibited reduced testosterone levels (Behne et al., 1996; Kaur and Bansal, 2004), a paucity of research exists investigating the effect of supplementing any form of Se other than sodium selenite on endocrine profiles, most likely also due to an economic standpoint.

One experiment I would like to conduct would be investigating the impact of Se supplementation on circulating levels of testosterone, LH, and FSH in the boar. I would really like to have a Se-deficient boar at puberty, if not several, to compare to supplemented counterparts. Based on our data presented herein, which involved feeding boars a basal diet that did not include any additional Se from 28 d of age through maturity, unsupplemented control boars were indeed Se-deficient during the nursery and grow-finish periods. The boars at the end of the grow-finish period were peripuberal. Sometime between the end of the grow-finish period, when the unsupplemented boars were Se-deficient, they became Se-adequate based on a blood Se measurement taken just prior to the semen quality experiments, which started when the boars were 15 mo of age. This does present a problem in keeping a Se-deficient boar model. Behne et al. (1996) can use Se-depleted rat models that have been depleted of all Se for several

generations, but I just do not see that being feasible from an animal welfare standpoint, especially when the longer gestation and growth to maturity periods of swine are considered.

As one can see, there are a multitude of areas that Se researchers can continue in the future. Adding Se to boar diets could be one way the producers could increase fertility of boars within the U.S. swine industry. The importance of Se to maintenance of fertility has been established, but much more research is needed in this area within the boar. There are additional means by which we could attempt to determine the molecular reason for the effects that are seen with Se deficiency or with Se supplementation, particularly in the characteristic midpiece abnormalities that are seen within spermatozoa. The data presented within the dissertation has shown that Se-supplementation, in the form of Sel-Plex, has been able to partially negate some of the decrease in sperm motility seen with a daily sperm collection challenge and a 10-d liquid sperm storage experiment at 18°C. Additional research is needed to see whether this relationship remains in cryopreserved boar spermatozoa.

CHAPTER 6

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