AN ABSTRACT OF THE THESIS OF

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Title: GLUTATHIONE PEROXIDASE AND SELENIUM DISTRIBUTION IN SEMEN AND REPRODUCTIVE TISSUES OF FARM ANIMALS

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Abstract approved: _____
Dr. Arthur S. Wu

This study was conducted to investigate the distribution of glutathione peroxidase (GSH-Px) and selenium in semen of the boar, bull, ram and stallion, and in the reproductive tissues of the boar, bull and ram. The effect of repeated noncryoprotective freeze-thawing on GSH-Px activity was also studied. Both hydrogen peroxide (H_2O_2) and tetra-butyl hydroperoxide (t-butyl O_2) were used as substrates for the enzyme.

Most of the GSH-Px activity in semen of the four species examined was associated with seminal plasma. GSH-Px activities in the spermatozoa of these species were comparatively very low. There were species and tissue differences in the distribution of GSH-Px in reproductive tissues. Determined per mg protein, GSH-Px activity was highest in the testis, whereas the Cowper's gland and ampulla showed the least enzyme activity. In general, GSH-Px levels in the reproductive tissues of the bull were

higher than, or comparable to, those of the ram. The reproductive tissues of the boar showed the least GSH-Px activity.

The GSH-Px determined in this study showed a similar magnitude of response to both H_2O_2 and t-butyl O_2 and had a high correlation with tissue and fluid selenium levels. This demonstrates that the GSH-Px found in semen and reproductive tissues of the boar, bull and ram is a selenium-dependent enzyme.

Repeated freezing (at -21°C) and thawing, in the absence of appropriate buffers, led to a decline in seminal plasma and reproductive tissue GSH-Px activities.

Selenium distribution in semen and reproductive tissues differed among the species studies. On the basis of selenium concentration per billion sperm cells, boar spermatozoa had the highest selenium concentration followed by those of the stallion, bull and ram in that order. Bull seminal plasma, however, had more selenium than that of any of the other three species. The testis and epididymis had the greatest concentration of selenium found in the reproductive tissues. Levels of selenium in semen and reproductive tissues were significantly higher ($P<0.05$) than those in blood. The implications of these findings, as well as the inter-relationship between selenium, GSH-Px and animal reproduction, are discussed.

Glutathione Peroxidase and Selenium Distribution In Semen and Reproductive Tissues of Farm Animals

by

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To God be the glory for the great things He has done!

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INTRODUCTION

The nature, function, and physiologic significance of glutathione peroxidase (GSH-Px) and the role of selenium in mammalian reproduction continue to be the subject of intensive research and debate. A GSH-Px (Glutathione H_2O_2 oxidoreductase, E.C.1.11.1.9) was first described by Mills and Randall (1958) in erythrocytes and, later, in other tissues. Evidence that peroxidase in erythrocytes, rather than catalase, protects hemoglobin from oxidation to methemoglobin by hydrogen peroxide (H_2O_2) was provided by Cohen and Hochstein (1963).

Hochstein and Utley (1968) showed that the peroxidase in the liver supernatant is also able to compete effectively with catalase for H_2O_2. Little and O'Brien (1968) have found that GSH-Px is probably responsible for most of the decomposition of lipid peroxide in the liver cells and may thus protect the cells from the deleterious effects of peroxides. O'Brien and Frazer (1966) demonstrated that catalase had no effect on the lipid peroxide produced in the liver. Christopherson (1968) has identified the lipid products formed by decomposition of lipid peroxide by GSH-Px and has further suggested that this enzyme may be able to break the autocatalytic chain reaction of lipid peroxidation and thus act as an antioxidant.

H_2O_2 is highly toxic to mammalian spermatozoa and studies have shown that oxygenation of semen is deleterious to sperm function (MacLeod, 1943; Van Demark et al., 1949). Moreover, spermine oxidase activity in seminal plasma is capable of producing H_2O_2 and the sperm cell itself can and does produce H_2O_2, presumably from oxidative deamination of certain amino acids (Tosic and Walton, 1950). Thus the existence of an intracellular, H_2O_2-removing mechanism such as that found in the erythrocyte would be beneficial to sperm survival which is of vital importance for high reproductive performance.

That selenium is a component of some GSH-Px (Flohé et al., 1973; Rotruck et al., 1973) is a well-documented fact. Some workers have shown that the specific activity of GSH-Px in rat (Scott, 1966) and chick (Bieri, 1959) tissues is a function of dietary selenium; that the activity of GSH-Px decreases to varying degrees in tissues from animals fed a basal low selenium diet, and that selenium supplementation leads to a significant increase in the activity of this enzyme (Chow and Tappel, 1974).

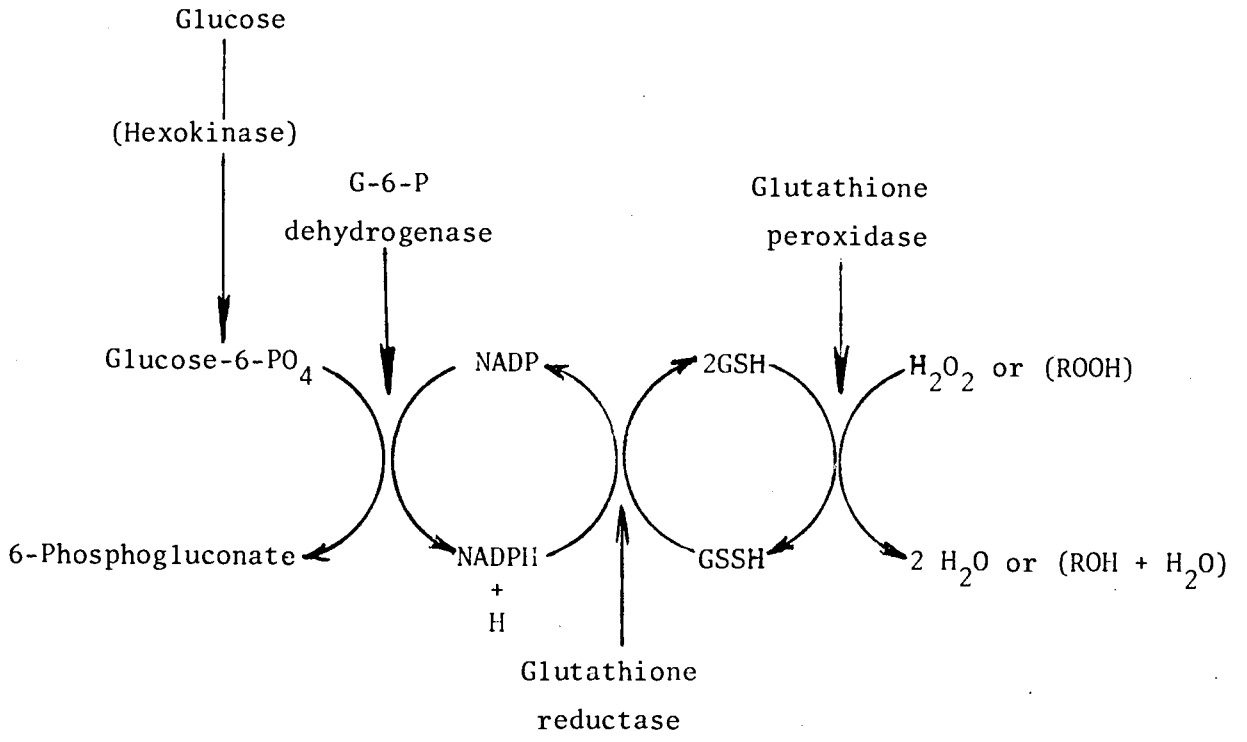
Although considerable work has been done to determine the distribution of GSH-Px and selenium in semen and non-reproductive tissues, very little systematic study has been carried out to determine their distribution in reproductive tissues of animals. The present study has, therefore, been undertaken to determine the distribution

of glutathione peroxidase and selenium in semen and reproductive tissues of farm animals as well as the effects of noncryoprotective freezing on glutathione peroxidase activity.

LITERATURE REVIEW

Glutathione as a Co-factor of GSH-Px

Glutathione is a tripeptide (γ-glutamylcysteinyl-glycine) and is widely distributed in living organisms. It is present often in high concentrations in plant and animal cells (Jocelyn, 1958). It participates in numerous metabolic processes, including the respiration of germinating seedlings and transport of amino acids (Meister, 1973). Glutathione is the essential co-factor of a number of enzymes including GSH-Px, and serves to preserve the sulfhydryl groups of proteins in the reduced state by means of disulfide interchange (Knox, 1960). The most well-established physiologic function of glutathione is that of protecting erythrocytes against oxidative damage (Cohen and Hockstein, 1963). Catalyzed by GSH-Px, glutathione reduces H_2O_2 to water and is itself converted to glutathione disulfide in the process. The glutathione disulfide thus formed is converted back to glutathione by glutathione reductase, as the following diagram illustrates:



Mechanism of peroxide destruction catalyzed by glutathione peroxidase (from Hoekstra, 1974). The abbreviations GSH and GSSH refer to reduced glutathione (γ-glutamylcysteinylglycine) and oxidized glutathione, respectively.

This "glutathione cycle" has been demonstrated to be an effective intracellular defense mechanism against a variety of cellular stresses (Flohé and Zimmerman, 1970; Kosower et al. 1971; Chow and Tappel, 1972).

Thiol compounds such as cysteine and glutathione have been shown to protect the motility and glycolytic activity of spermatozoa in vitro against the inhibitory actions of heavy metal ions (MacLeod, 1951; White, 1955) and oxidizing agents (VanDemark et al., 1949; Wales et al., 1959). Other compounds which may be effective in this regard are ergothioneine and ascorbic acid, present in the

seminal plasma of some animal species (Mann, 1964). Oxidation of intracellular thiol groups occurs with senescence and during aging of sea urchin spermatozoa, when glutathione content decreases dramatically (Backstrom, 1958).

Distribution of GSH-Px in Mammalian Semen and Reproductive Organs

GSH-Px activity has been found in the semen of several species including the ram, dog, human, goat (Li, 1975) and bull (Brown et al., 1977; Smith et al., 1979; Pond, 1980). GSH-Px activity has been demonstrated to be associated with seminal plasma and not spermatozoa in the human (Li, 1975), ram (Pond, 1980), bull (Brown, et al., 1977; Smith et al., 1979; Pond, 1980) and rat (Calvin and Cooper, 1979). Pond (1980) found no GSH-Px activity in ram spermatozoa; Li (1975), however, reported some activity of this enzyme in the sperm cells of the ram.

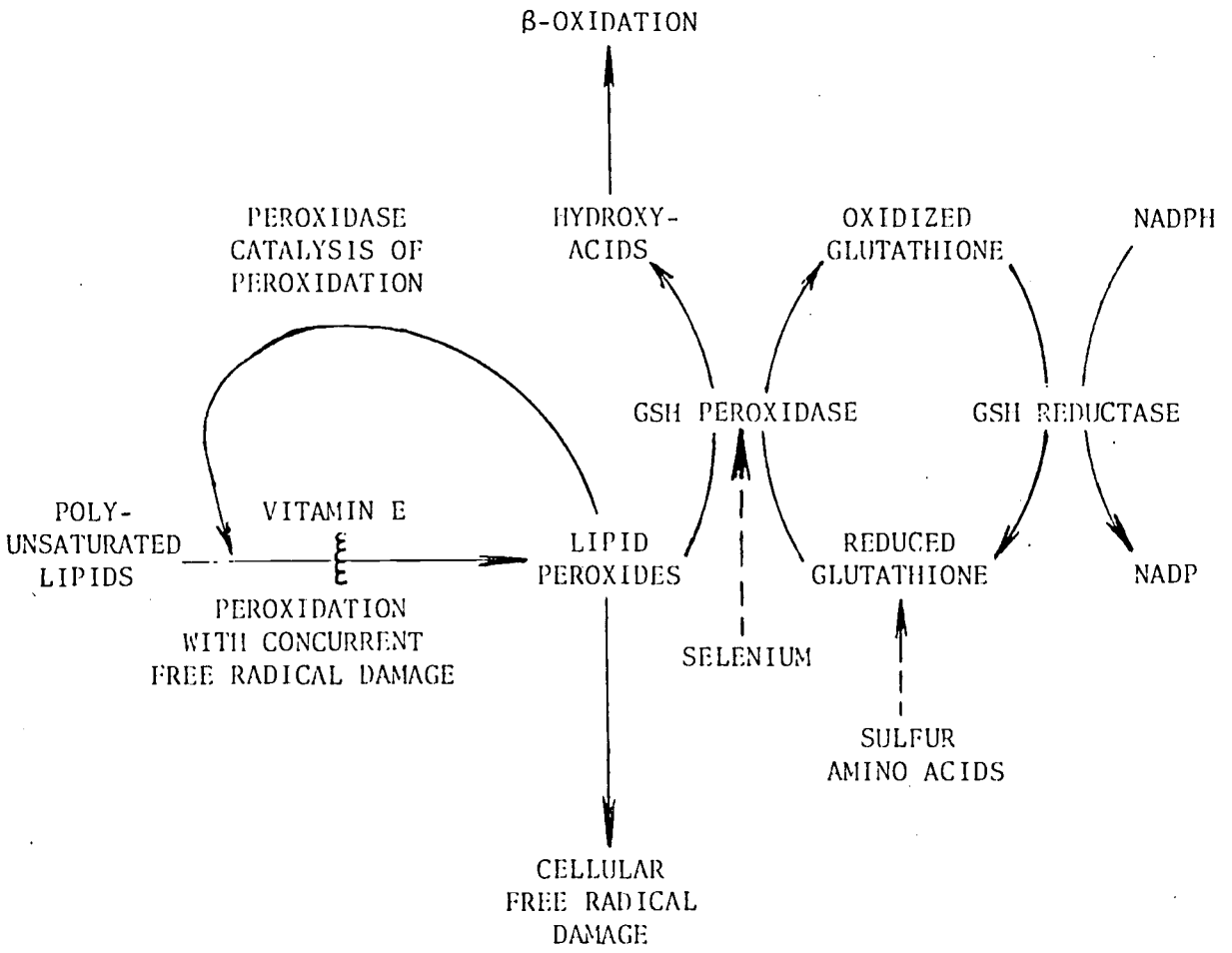
Significant levels of GSH-Px have been found in rat testis (Chow and Tappel, 1974) and vesicular glands of the bull and ram (Pond, 1980); however, apart from these reports, very little information exists in the literature as to the nature of GSH-Px distribution in mammalian reproductive tissues.

Selenium as a Component of GSH-Px

The first evidence that GSH-Px may require selenium for its normal activity was presented by Rotruck et al. (1973) on work with rat erythrocytes. Subsequently, when the enzyme was isolated from cattle (Flohé et al., 1973), sheep (Oh et al., 1974) or humans (Awasthi et al., 1975) and purified, it was shown to be indeed a selenoenzyme containing about 4 gram atoms of the element per mole of enzyme. Addition of selenium to diets for rats (Chow and Tappel, 1974; Smith et al., 1974; Hoekstra, 1974), chicks (Noguchi et al., 1973; Omaye and Tappel, 1974) and sheep (Godwin et al., 1975; Oh et al., 1976; Whanger et al., 1977) results in a significant increase of their tissue glutathione peroxidase activities.

Even though it has been demonstrated that both selenium and vitamin E are involved in the prevention of nutritional muscular dystrophy in the chick (Calvert et al., 1962) and liver necrosis in rats (Schwartz and Foltz, 1957), Whanger et al. (1977) have shown that vitamin E has no influence on either blood selenium levels or upon tissue GSH-Px activity. These workers found a positive correlation between erythrocyte GSH-Px levels and blood selenium levels. Presented below is a diagram illustrating what Chow and Tappel (1974) proposed as possible interactions among selenium (as a component of GSH-Px), vitamin E and the sulfur amino acids in the

inhibition of lipid peroxidation damage to tissues:



Interactions among selenium, vitamin E and the sulfur amino acids in inhibition of lipid peroxidation damage.

Distribution and Function of Selenium in Spermatozoa

Wu et al. (1969, 1971) working with laboratory rats, reported that selenium deficiency affected male reproductive functions. Their results showed that selenium deficiency in male offspring from selenium-deficient female rats

produced sperm with impaired motility and a unique type of midpiece abnormality. It was observed that dietary supplementation with α-tocopherol or other antioxidants did not alleviate these symptoms of selenium deficiency (Wu et al., 1973), suggesting that selenium plays a specific role in maintaining the structural and functional integrity of the sperm cell. Later, electron microscopy revealed damage in selenium-deficient rats to be localized in the membrane system near the sperm midpiece (Wu et al., 1979).

Most of the selenium in rat liver mitochondria has been reported to be in the form of GSH-Px and is released from the mitochondria during swelling induced by reduced glutathione (Neubert et al., 1962). In bull spermatozoa, it has been demonstrated (Pallini and Bacci, 1979) that selenium is selectively localized in the mitochondria where it is bound to a structural polypeptide and is believed to function in the stabilization of the outer mitochondrial membrane. Smith et al. (1979) found GSH-Px activity only in seminal plasma of the bull and postulated a structural role for selenium in bull spermatozoa.

Calvin (1978) found that, following an intratesticular injection of ^{75}Se, the label was localized primarily in the tail keratin of rat spermatozoa. He identified a 17,000 dalton polypeptide with which selenium was associated. Calvin's work was corroborated by later

investigations carried out by Niemi et al. (1981) who reported that 85% of the ^{75}Se found in the bovine spermatozoa was associated with a protein similar in size (21,500 daltons for the bull vs 17,000 daltons for the rat) to the protein in rat sperm tails described by Calvin (1978). Calvin and Cooper (1979) proposed that the ^{75}Se labeled polypeptide was associated with the outer mitochondrial membranes and in some way essential for the assembly of the mitochondrial sheath. They doubted that this polypeptide was a subunit of GSH-Px.

Selenoproteins other than GSH-Px have been found. Two microbial selenoenzyme systems, glycine reductase of Clostridia and bacterial formate dehydrogenase, have been described by Stadtman (1974). Whanger et al. (1973) isolated a selenium-containing protein from heart and semitendinosus muscles of lambs, possibly a selenium-containing cytochrome which may participate in oxidation-reduction reactions, though its catalytic functions are not yet determined. Selenium is also associated with pyruvate oxidation in liver preparations, which suggests that it may function in oxidative processes of the tricarboxylic acid cycle in vivo (Bull and Oldfield, 1967).

Gunn and Gould (1970) suggested that ^{75}Se is incorporated by the early spermatid or secondary spermatocyte and that ^{75}Se is not directly incorporated by spermatozoa residing in the epididymis. The incorporation of

selenium by the spermatid may reflect a requirement of selenium for normal spermiogenesis (Smith et al., 1979).

Distribution of Selenium in Seminal Plasma and Reproductive Organs

Smith et al. (1979) have shown that, following a single intravenous injection of ^{75}Se, this isotope is concentrated in bovine semen, with the greater portion of it being incorporated in the seminal plasma. These workers found ^{75}Se in seminal plasma at levels greatly exceeding blood ^{75}Se content and suggested that ^{75}Se is accumulated and secreted by one or more of the accessory glands. The majority of the ^{75}Se in seminal plasma was found to be associated with protein.

Tissue retention data (Smith et al., 1979) indicated that the prostate and vesicular glands of the bull contained levels of ^{75}Se exceeded only by the testis, epididymis and kidney, and it was suggested that these glands were responsible for most of the ^{75}Se in bovine seminal plasma. Of all the accessory glands examined, Cowper's glands and ampulla had the least amount of ^{75}Se. Pond (1980) reported similar patterns of ^{75}Se distribution in ram reproductive tissues--i.e., the testis incorporated the highest amount of ^{75}Se followed by the vesicular glands and Cowper's glands in that order.

The possibility that species differences exist in

the pattern of selenium distribution in the reproductive tissues of various animals cannot be ruled out since Hansson and Jacobsson (1966), using whole body autoradiography in mice, found that the vesicular glands, and not the testes, showed the highest concentration of ^{75}Se four days after injection.

MATERIALS AND METHODS

Experiment 1

A. GSH-Px Activity in Seminal Plasma and Spermatozoa

Five animals from each of the four species: bull, ram, boar and stallion, were used in this study. Only animals that appeared healthy and devoid of any physical defects were used. Semen ejaculates were collected over a period of eight weeks, using an artificial vagina (for the boars and stallions) or an electroejaculator (for the rams and bulls). Each ejaculate was evaluated for volume, sperm concentration and motility immediately after collection.

Spermatozoa and seminal plasma were separated by centrifugation at 5,000 x g for 10 minutes. Aliquots of the seminal plasma fraction of each sample were immediately assayed for GSH-Px activity. Sufficient amounts of seminal plasma from each sample were also kept in vials at -21°C for later assays of GSH-Px activity and selenium content. The spermatozoa were washed with 0.9% physiological saline and centrifuged to remove residual seminal plasma. To insure complete or nearly complete rupturing of the sperm membranes, the washed spermatozoa were resuspended in double distilled water, frozen at -21°C for 24 hours, thawed and centrifuged again at 5,000 x g

for 10 minutes. The supernatants obtained after final centrifugation were assayed for GSH-Px activity.

B. Effect of Freeze-Thawing on GSH-Px Activity in Seminal Plasma

To determine the effect of noncryoprotective freeze-thawing on seminal plasma GSH-Px activity, samples of seminal plasma were frozen at -21°C and thawed after 5 days of freezing by placing the samples in an ice-bath for 30 to 45 minutes. Aliquots were then removed and assayed for GSH-Px activity while the rest of the samples were returned to the freezer. This procedure was repeated at 5-day intervals for 50 days.

C. Technique of GSH-Px Assay

GSH-Px activities were determined by the coupled enzyme procedure of Paglia and Valentine (1967) as modified by Whanger, et al. (1977). The enzyme was assayed in a volume of 0.1 ml of 0.05 M phosphate buffer, pH 7, containing 0.05 M EDTA, 0.24 mg sodium azide, 0.67 units of glutathione reductase, 0.233 mg NADPH, 1.533 mg reduced glutathione and either 0.07 M H_2O_2 or 2.5 mM tetra-butyl hydroperoxide (t-butyl O_2). All components, except H_2O_2 and t-butyl O_2, were mixed together in sufficient amounts for the number of assays needed for each set of determinations. Both H_2O_2 and t-butyl O_2 were used as substrates for the GSH-Px assays in the present study to

determine the difference, if any, in GSH-Px response to H_2O_2 and t-butyl O_2. The sample (0.1 ml) was added to the cuvette, followed by the reaction mixture (0.8 ml). The reaction was initiated by adding 0.1 ml of H_2O_2 or t-butyl O_2. GSH-Px activities were then measured spectrophotometrically at 340 nm, using a Hitachi 100-80A spectrophotometer. The blank cuvette contained distilled water instead of the test samples, and the values obtained for the blanks were subtracted from those of the test samples before the enzyme units were calculated.

Ten measurements were made for each sample and GSH-Px activity was expressed as nanomoles NADPH oxidized per minute either per mg of protein or per ml of the original sample. The chemicals used were obtained from Sigma Chemical Company. All assays were carried out at 28°C.

Aliquots of the sperm supernatants and seminal plasma were also assayed for protein content by the method described by Lowry et al. (1951), using bovine serum albumin as standards.

Experiment 2

A. GSH-Px Activity in Reproductive Tissues

Tissues of the reproductive tracts of the boar, bull and ram (10 animals from each species) were obtained from various slaughter plants in Oregon. The specimens arrived in the laboratory in ice chests at about 0°C.

Testes, epididymides, vesicular glands, ampullae, prostate and Cowper's glands were dissected out, trimmed and rinsed free of blood. Fluid was squeezed out from the testes, epididymides and vesicular glands by gently pressing the tissues with a spatula, and the fluid so obtained was assayed separately for GSH-Px activity and protein content. A known weight (5-10 g wet weight) of each of the reproductive tissues was also homogenized in 5 volumes of 0.1 M phosphate--10% sucrose buffer (pH 7.0), using a Sorvall Omni-mixer. The homogenates were centrifuged at 161,000 x g at 4°C for 90 minutes. Aliquots of the cytosol of each of these tissues were assayed for both GSH-Px activity and protein content according to methods previously described.

B. Effect of Freeze-Thawing on GSH-Px Activity in Reproductive Tissues

Sufficient amounts of the cytosol of each of the reproductive tissue homogenates were kept in vials and frozen at -21°C. The samples were thawed after 5 days of freezing by placing them in an ice-bath for 30 to 45 minutes, after which aliquots were removed for GSH-Px assays. The rest of the samples were re-frozen and the procedure was repeated at 5-day intervals for 50 days to determine the effect of repeated noncryoprotective freeze-thawing on reproductive tissue GSH-Px activity.

Experiment 3 Selenium Distribution in Semen and Reproductive Tissues

The seminal plasma, spermatozoa and known weights (0.5 - 1.0 g wet wt.) of various reproductive tissues were each analyzed fluorometrically for selenium content according to the method of Brown and Watkinson (1977). To determine the selenium status of each of the animals used in the present study, blood was collected via jugular puncture, using heparinized vacutainers and analyzed for selenium content along with the other tissue samples. All determinations were in duplicates.

Statistical Analysis

All data obtained in this study were analyzed using such standard statistical procedures as linear regression and factorial analysis of variance according to Steel and Torrie (1980).

RESULTS

Experiment 1

A. GSH-Px Activity in Seminal Plasma and Spermatozoa

GSH-Px activities, obtained with either H_2O_2 or t-butyl O_2 as substrate, in seminal plasma and spermatozoa of the boar, bull, ram and stallion are presented in Figure 1.

When enzyme activity was expressed as nanomoles NADPH oxidized per minute per ml of seminal plasma, bull seminal plasma had the highest GSH-Px activity. This was followed, in a descending order, by the seminal plasma of the stallion, ram and boar (Figure 1; see also appendix Table 1). When GSH-Px activity was expressed as nanomoles NADPH oxidized per minute per mg protein, however, the activity of this enzyme in bull seminal plasma was comparable to that of the ram (Table 1). Seminal plasma of these two species showed about twice the level of GSH-Px (per mg protein) found in that of the stallion. Boar seminal plasma showed the least GSH-Px activity.

The pattern of GSH-Px activity in the spermatozoa of these four species (Figure 1) was quite different from that found in the seminal plasma. Ram spermatozoa showed the highest GSH-Px activity per ml of semen, with bull, stallion and boar spermatozoa following in that order.

This order was retained when enzyme activity was determined per mg of sperm protein (Table 2). However, expressed per billion sperm cells, GSH-Px activity was significantly higher ($P<0.01$) in stallion spermatozoa than in those of the boar, bull and ram which were comparable (Figure 2; Table 3).

B. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Seminal Plasma

Activity of GSH-Px in seminal plasma showed a general decline with repeated freeze-thawing (Figure 3). Ram and stallion seminal plasma showed the greatest reduction, 74%, in enzyme activity (determined on a per mg protein basis). Following closely was bull seminal plasma, with a reduction in GSH-Px activity of about 50%. Levels of GSH-Px in boar seminal plasma, however, remained fairly constant throughout the observation period of 50 days.

Experiment 2

A. GSH-Px Activity in Reproductive Tissues

Figures 4-8 show the activities of GSH-Px in the reproductive tissues of the boar, bull and ram expressed as nanomoles NADPH oxidized/min/mg protein. In each of the three species, the testis (testicular parenchyma and fluid) showed the highest GSH-Px activity followed by the epididymis and vesicular gland. GSH-Px activities in bull testis and vesicular glands were similar to those of corresponding tissues of the ram (Figures 4 and 6). These

activities were, however, significantly higher ($P<0.01$) than those in the testis and vesicular gland of the boar. Bull epididymis showed a higher ($P<0.05$) GSH-Px activity than the epididymis of the ram whose enzyme activity was significantly higher ($P<0.01$) than that of the boar (Figure 5). The Cowper's gland (Figure 8) of the ram contained a higher ($P<0.05$) level of GSH-Px than that of the bull; however, levels of this enzyme in the ampullae of the two species were similar (Figure 7).

B. Effect of Repeated Freeze-Thawing on GSH-Px Activities in Reproductive Tissues

As was the case with seminal plasma, there was a general decline in GSH-Px activities with repeated freezing (at -21°C) and thawing of reproductive tissue cytosols and fluids (Figures 9-13). The reduction in GSH-Px activities in the reproductive tissues of the bull and ram tended to be more pronounced than in those of the boar which remained fairly constant for the most part of the observation period. The GSH-Px activities obtained for each of the three species during the 50-day observation period are presented in Table 13.

Factorial analysis of all the samples examined in this study (spermatozoa, seminal plasma and reproductive tissues) indicated that there were no significant differences in the GSH-Px response to H_2O_2 and t-butyl O_2.

Experiment 3

A. Blood Selenium

The mean (±SEM) selenium levels in blood were respectively 0.22±0.03, 0.19±0.03, 0.16±0.04 and 0.05±0.01 µg/ml for the boars, bulls, rams and stallions used in the first experiment. For the boars, bulls and rams used in the second experiment, the blood selenium levels were 0.20±0.02, 0.21±0.05 and 0.15±0.04 µg/ml respectively.

B. Selenium Distribution in Semen

Spermatozoa of the boar, bull, ram and stallion contained respectively, 64.3, 46.7, 60.6 and 34.5 per cent of the total selenium content of semen; whereas the concentration of this element in the seminal plasma of these four species represented, respectively, 35.7, 55.3, 46.4 and 65.5 per cent of the total semen selenium levels (Figure 14). The concentrations of selenium in seminal plasma and spermatozoa, expressed as µg selenium/ml of semen, are presented in Figure 15. On the basis of selenium concentration per billion sperm cells, the boar had a significantly greater ($P<0.05$) amount of selenium than any of the other three species (Figure 16; Table 15).

C. Selenium Distribution in Reproductive Tissues

In the boar and ram, the testis had the highest level of selenium (0.65 and 1.4 µg/g testicular tissue respectively) but in the bull, selenium concentration

(1.83 µg/g testicular tissue) was greatest in the epididymis. The ampulla and Cowper's gland had the least selenium content (Figure 17).

Factorial analysis of variance showed that bull reproductive tissues had significantly higher ($P<0.05$) selenium levels than those of the ram whose selenium levels were, in turn, significantly higher ($P<0.05$) than those of the reproductive tissues of the boar. Linear regression analyses showed selenium levels of reproductive tissues and their GSH-Px activities (expressed as nmoles NADPH oxidized per minute per mg protein) to be significantly correlated ($r = 0.89$, $P<0.01$ for the boars; $r = 0.78$, $P<0.05$ for the bulls; $r = 0.87$, $P<0.01$ for the rams).

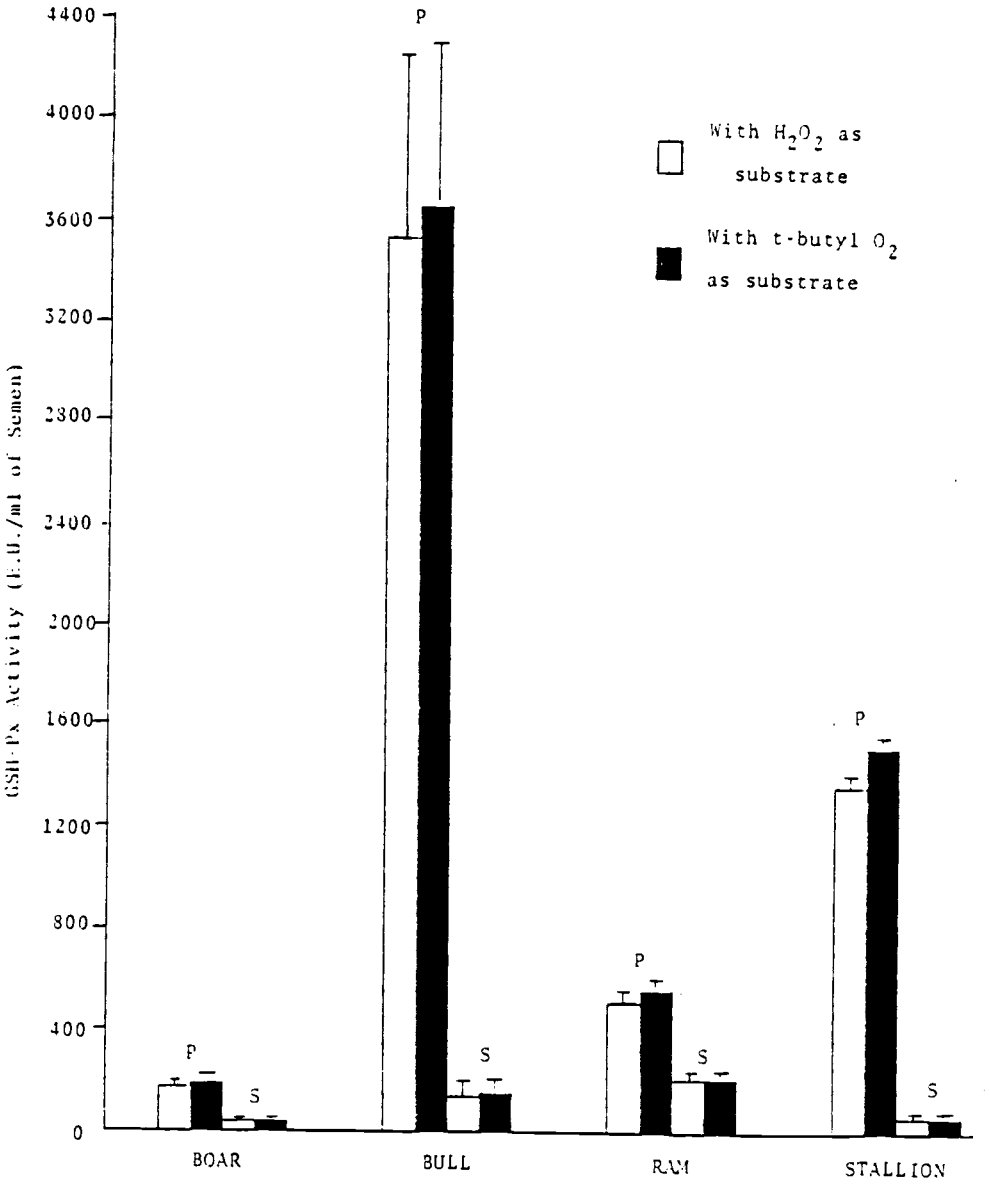


Figure 1. GSH-Px Distribution in Semen (Means ± SEM; P = Seminal Plasma; S = Spermatozoa).

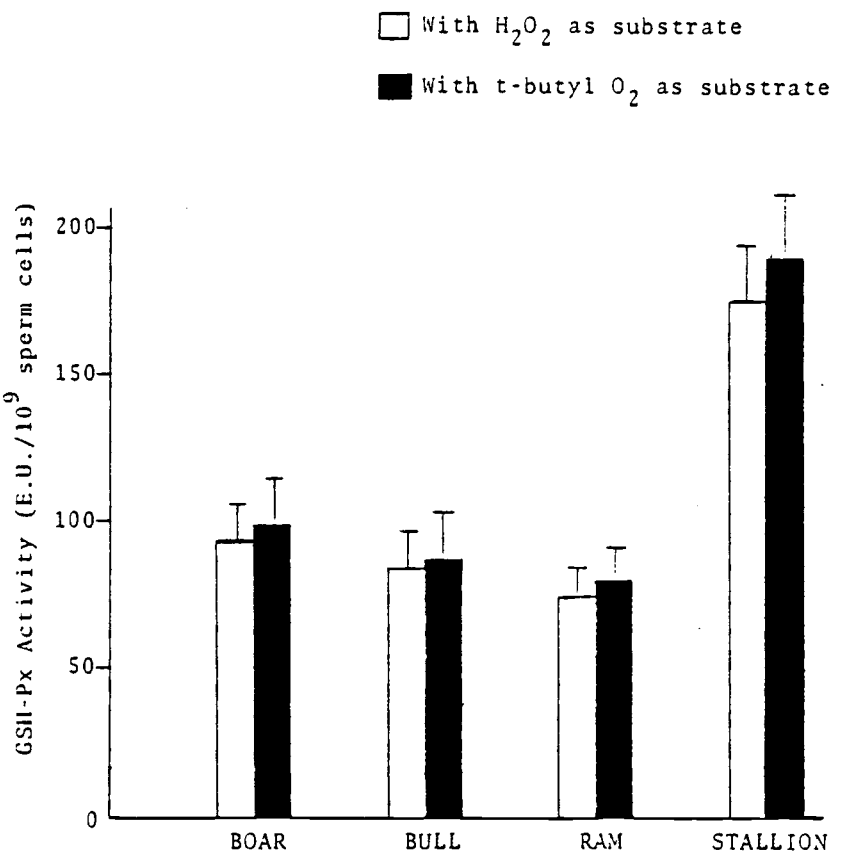


Figure 2. GSH-Px Activity in Spermatozoa (Means ± SEM).

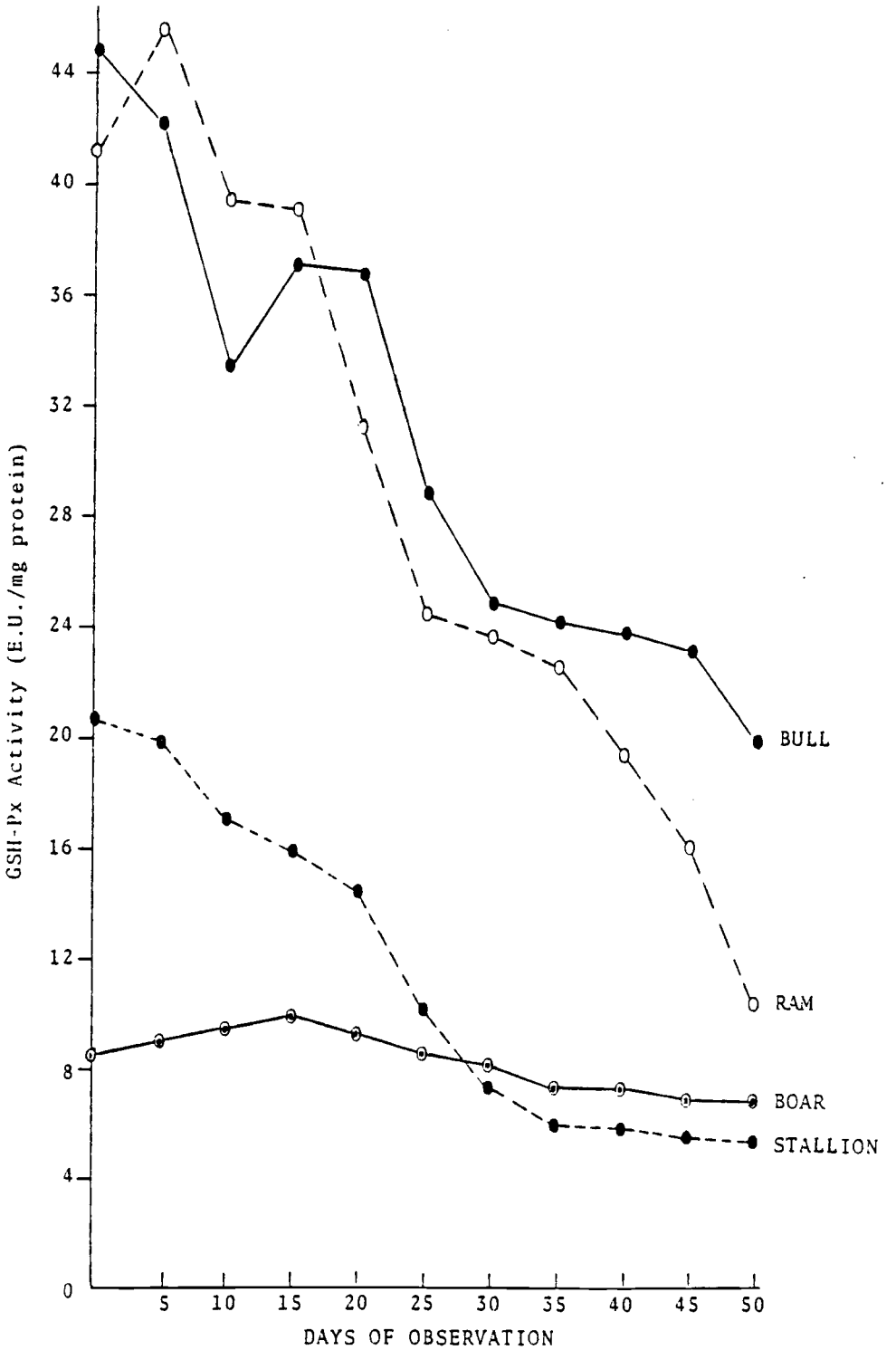


Figure 3. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Seminal Plasma.

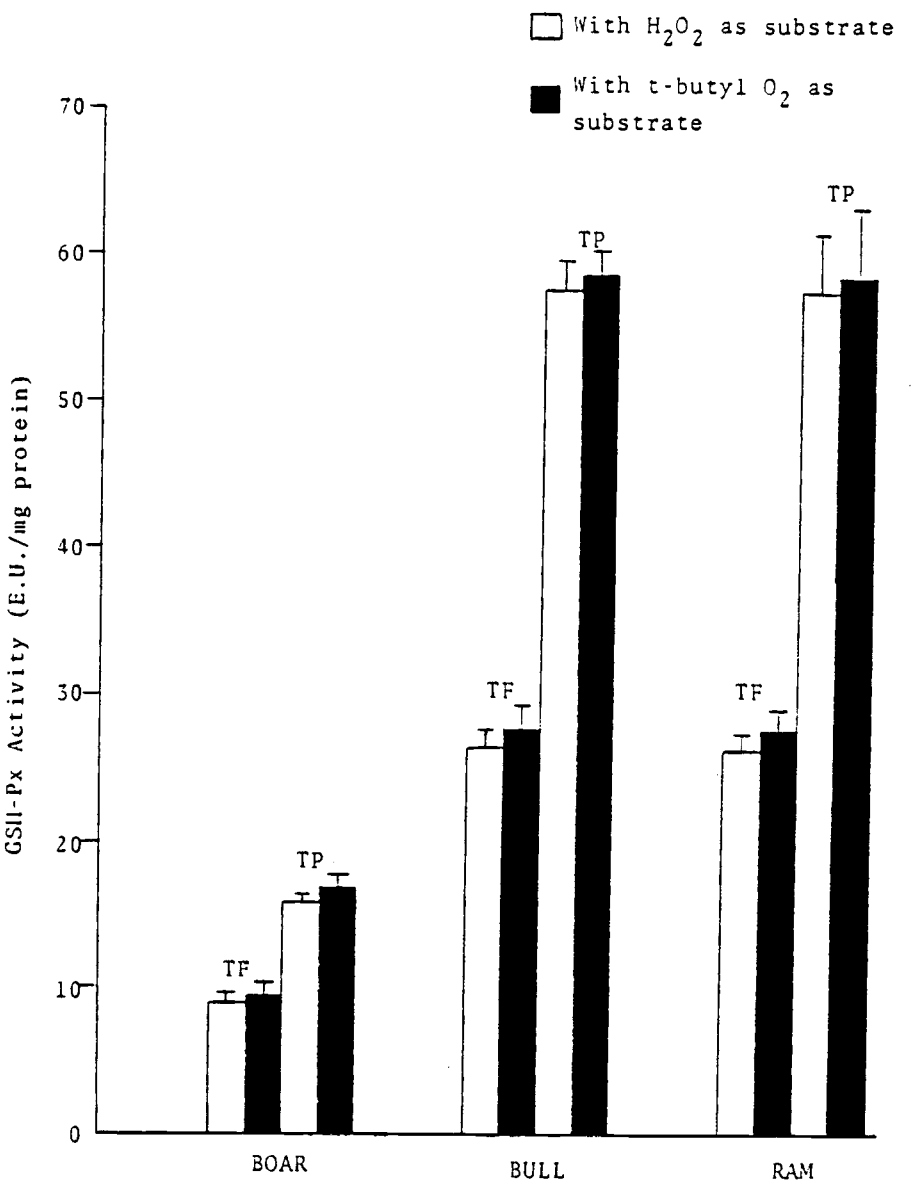


Figure 4. GSH-Px Activity in Testis (Means ± SEM; TP = Testicular Parenchyma; TF = Testicular Fluid).

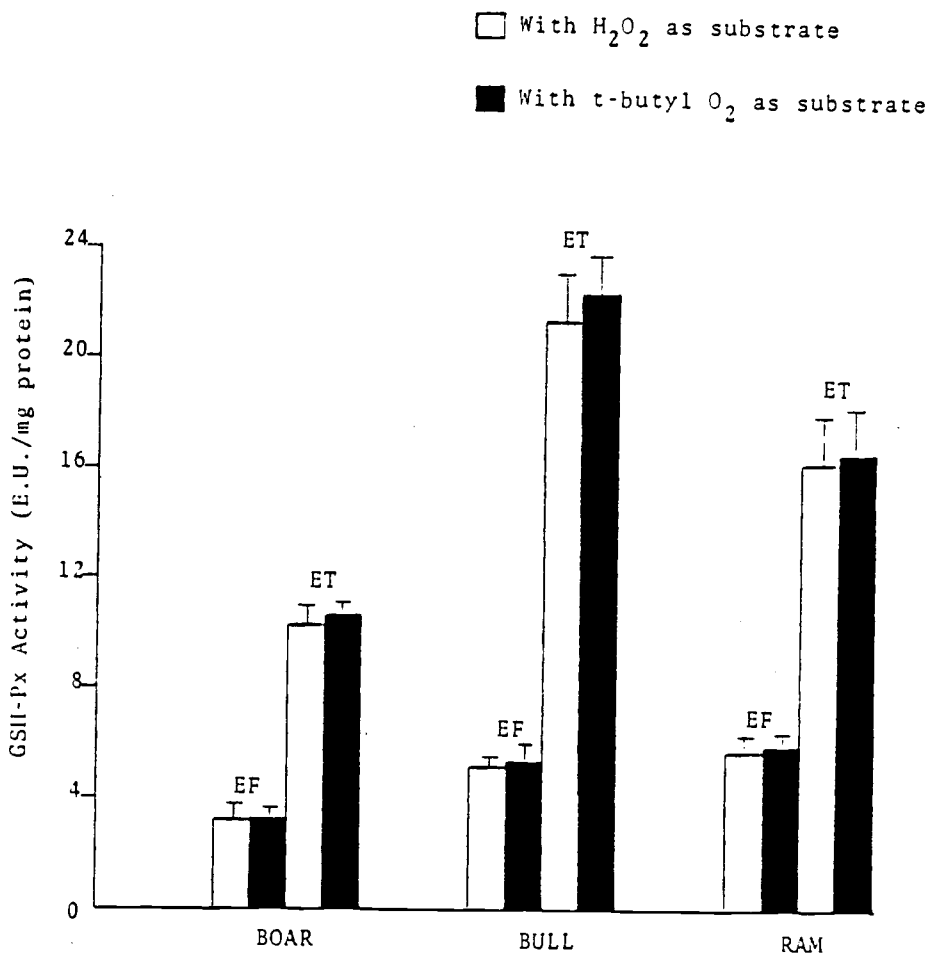


Figure 5. GSH-Px Activity in Epididymis (Means ± SEM; ET = Epididymal Tissue; EF = Epididymal Fluid).

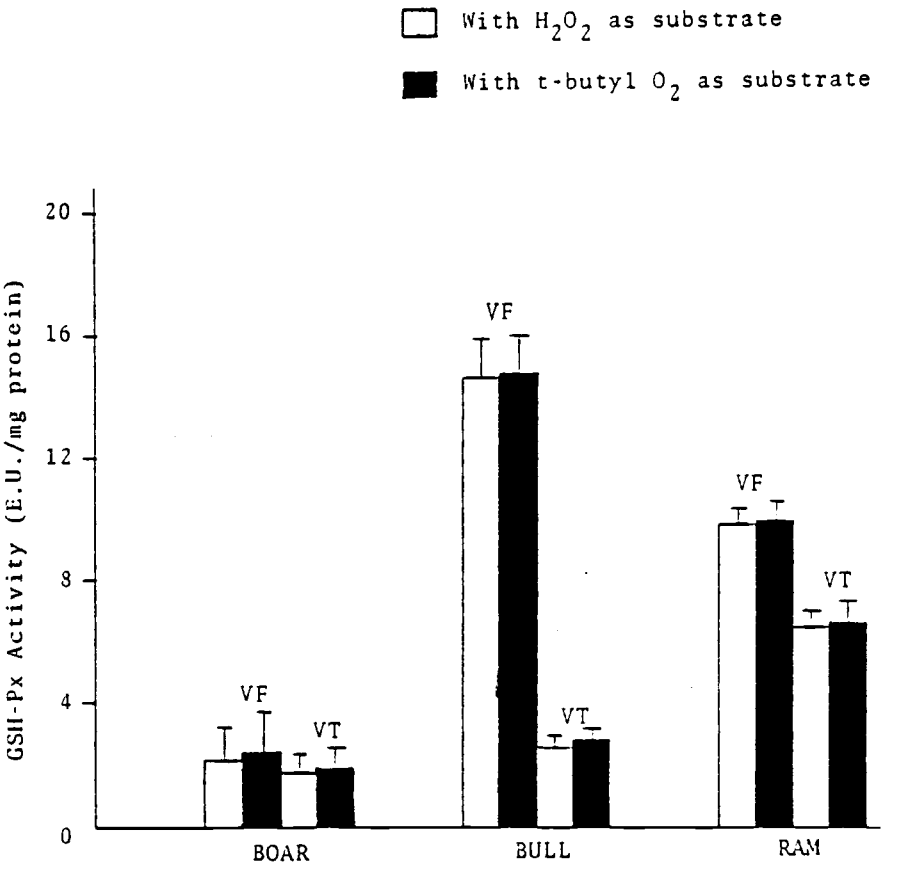


Figure 6. GSH-Px Activity in Vesicular Gland (Means ± SEM; VT = Vesicular Tissue; VF = Vesicular Fluid).

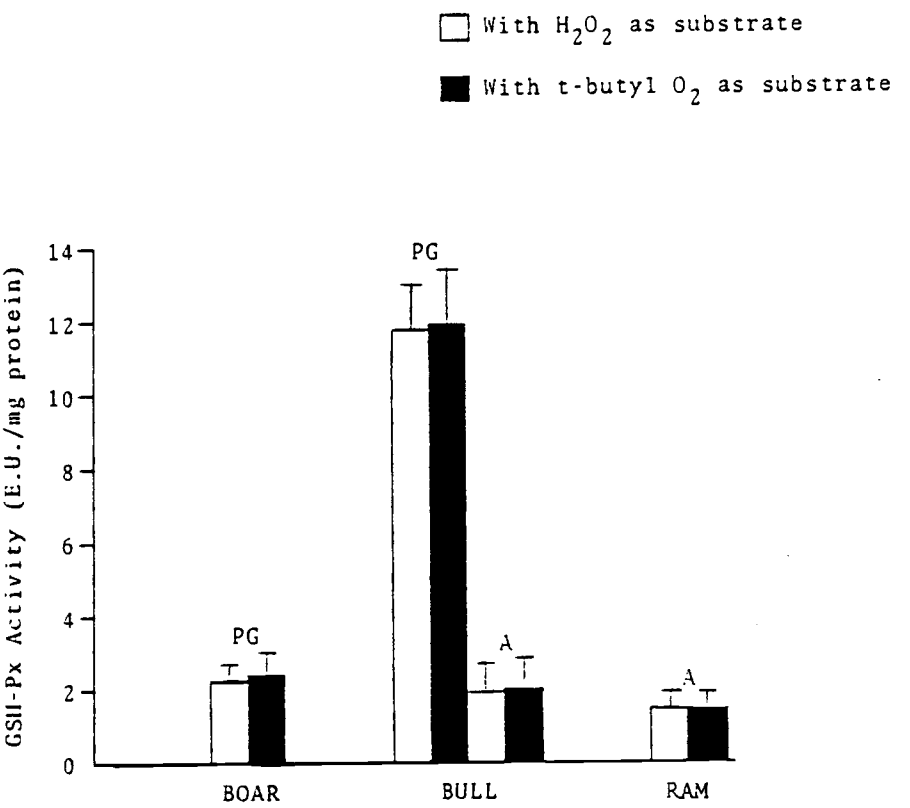


Figure 7. GSH-Px Activity in Prostate Gland (PG) and Ampulla (A) (Means ± SEM).

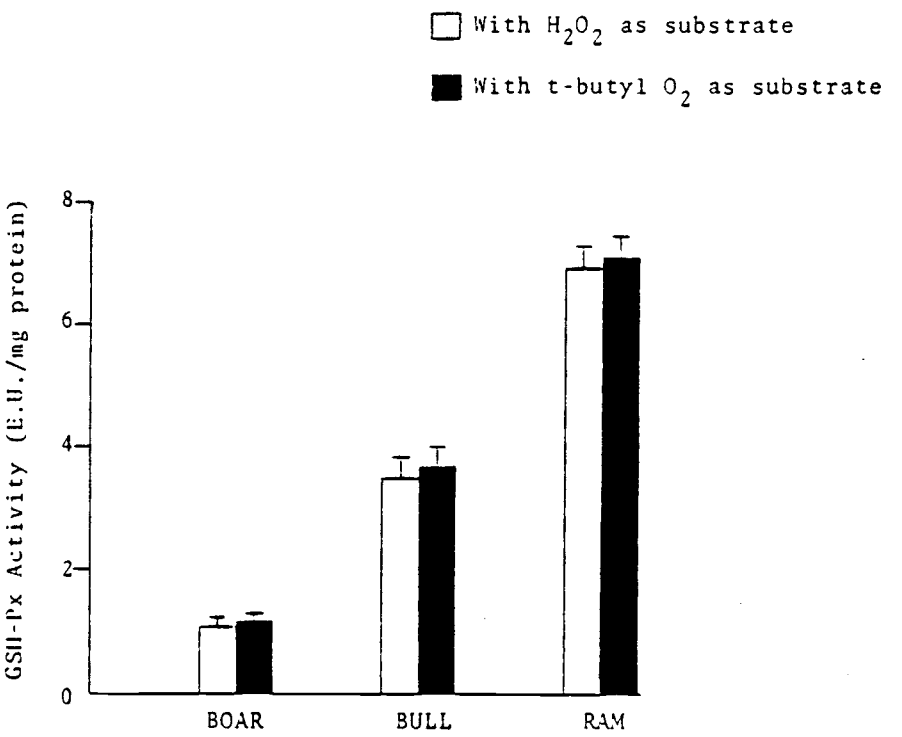


Figure 8. GSH-Px Activity in Cowper's Gland (Means ± SEM).

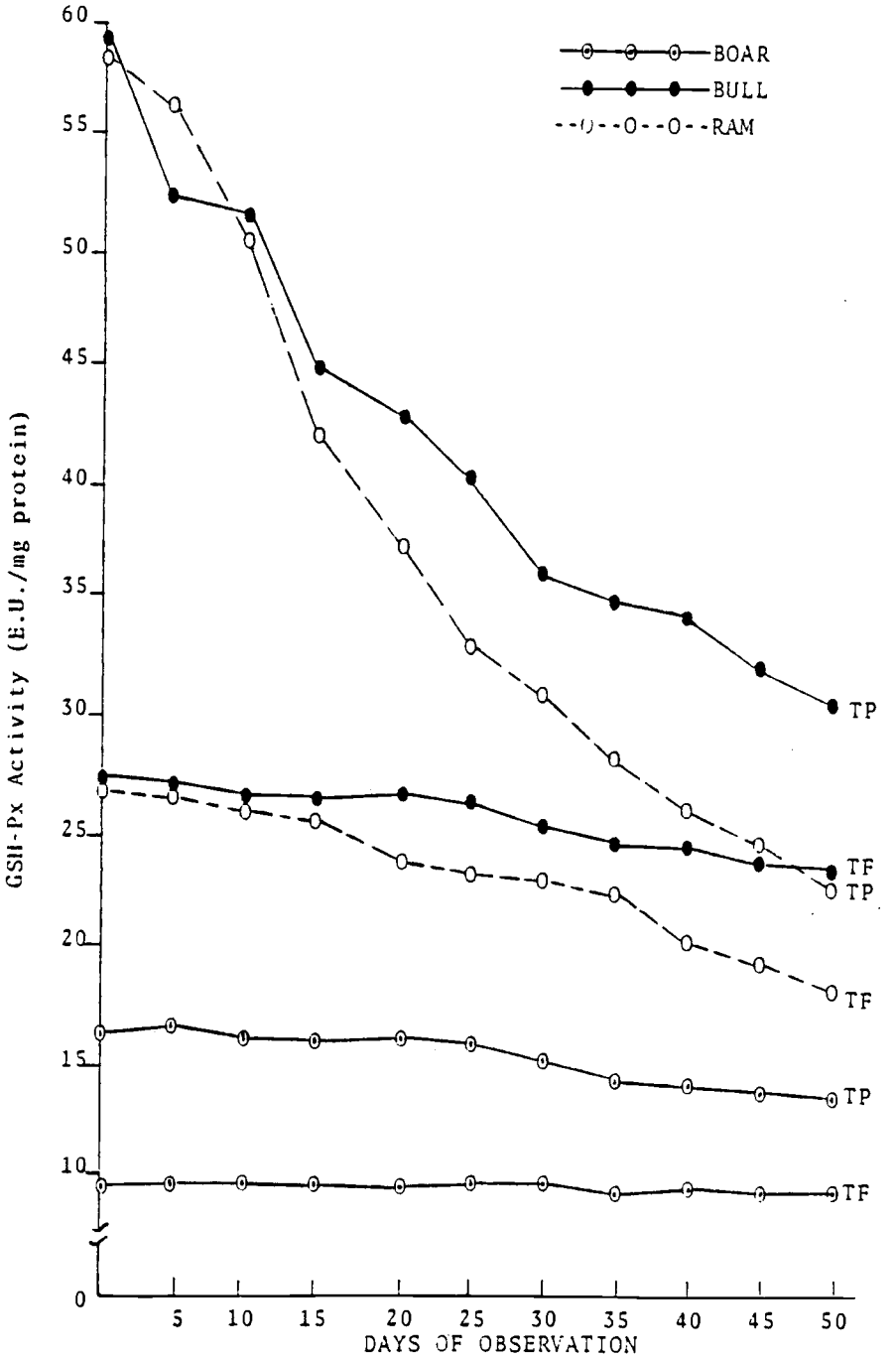


Figure 9. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Testis (TP = Testicular Parenchyma; TF = Testicular Fluid).

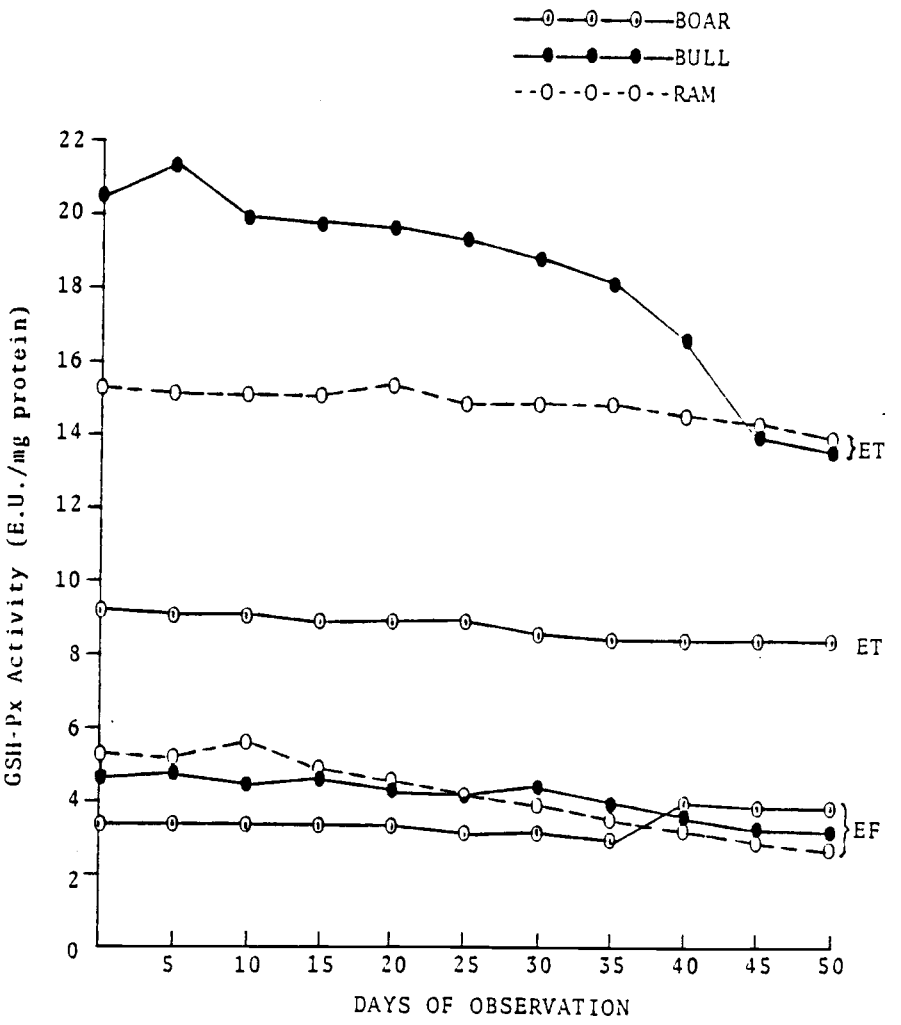


Figure 10. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Epididymis (ET = Epididymal Tissue; EF = Epididymal Fluid).

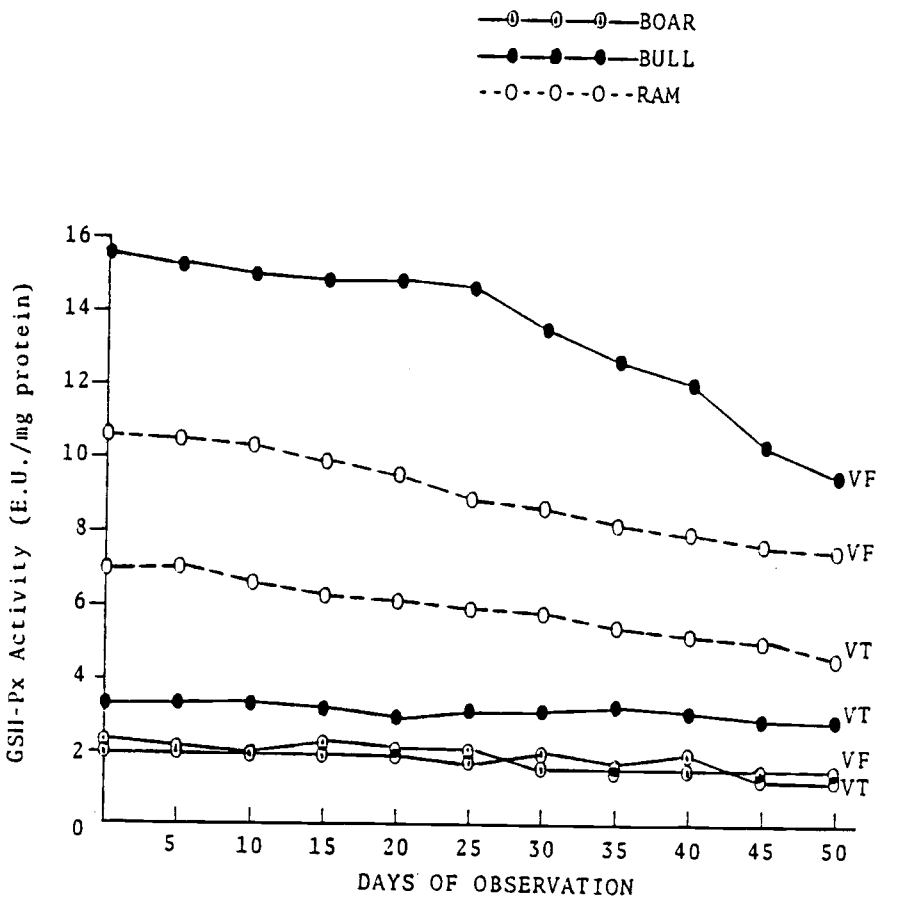


Figure 11. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Vesicular Gland (VT = Vesicular Tissue; VF = Vesicular Fluid).

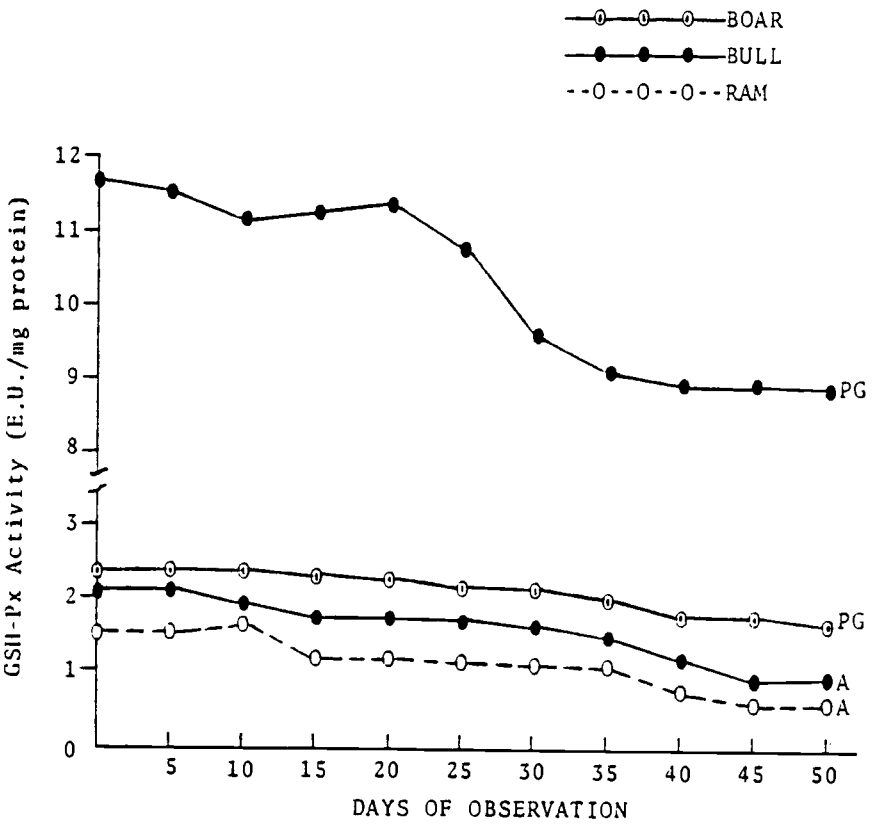


Figure 12. Effect of Repeated Freeze-Thawing on GSH-Px Activities in Prostate Gland (PG) and Ampulla (A).

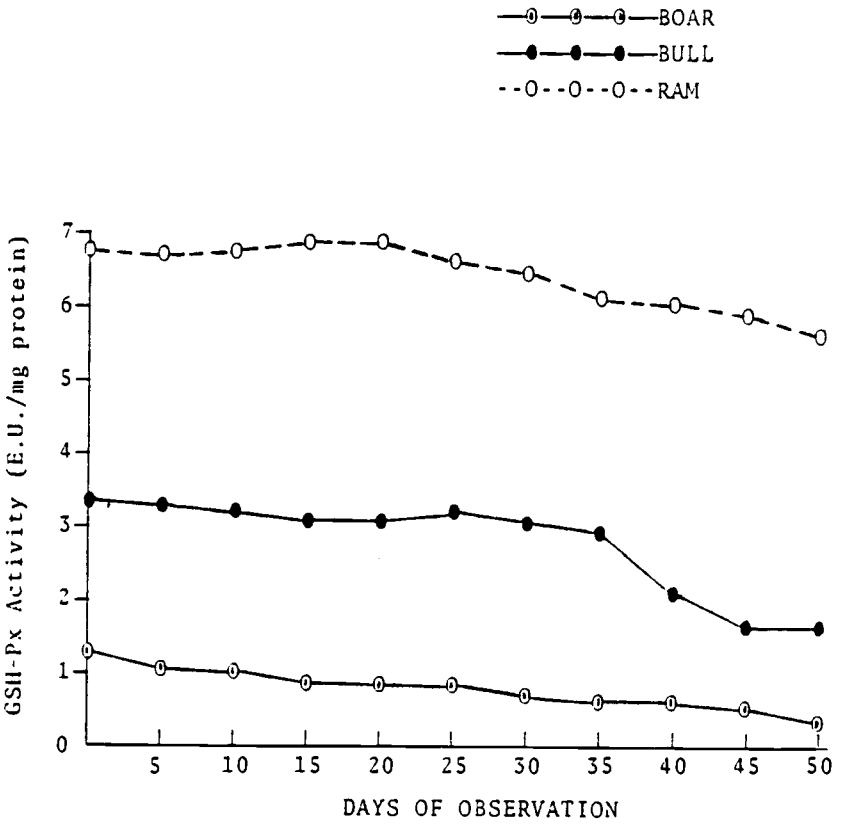


Figure 13. Effect of Repeated Freeze-Thawing on GSH-Px Activity in Cowper's Gland.

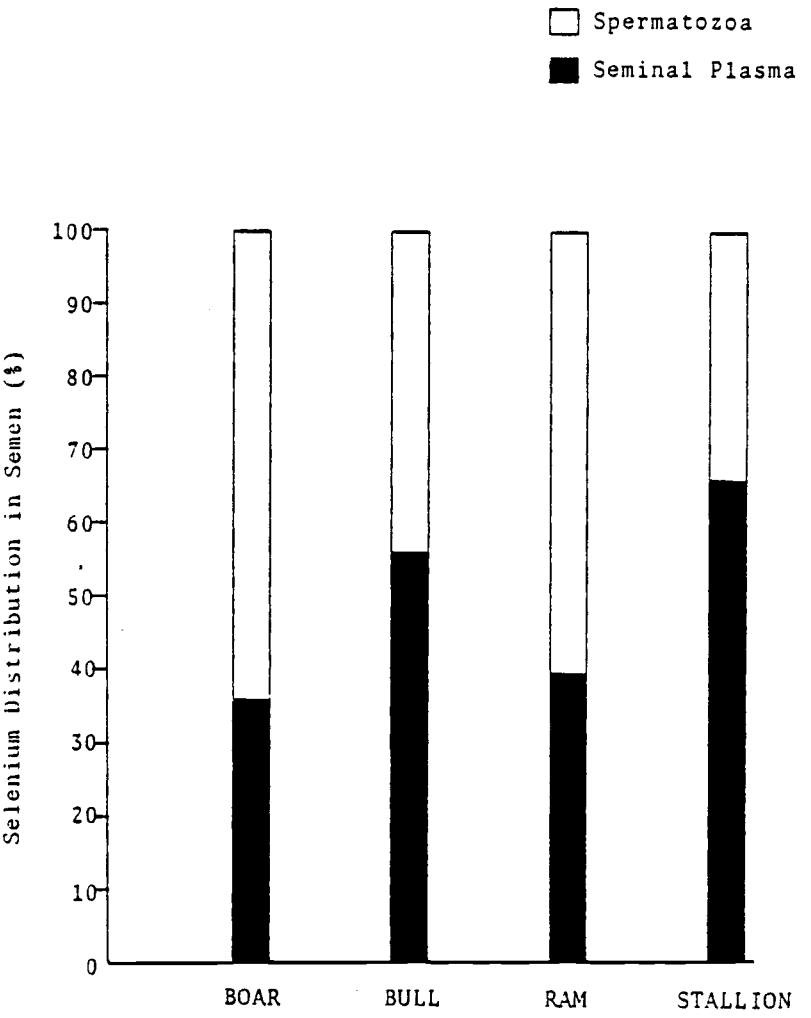


Figure 14. Selenium Content of Spermatozoa and Seminal Plasma Expressed as Per Cent of Total Semen Selenium Levels.

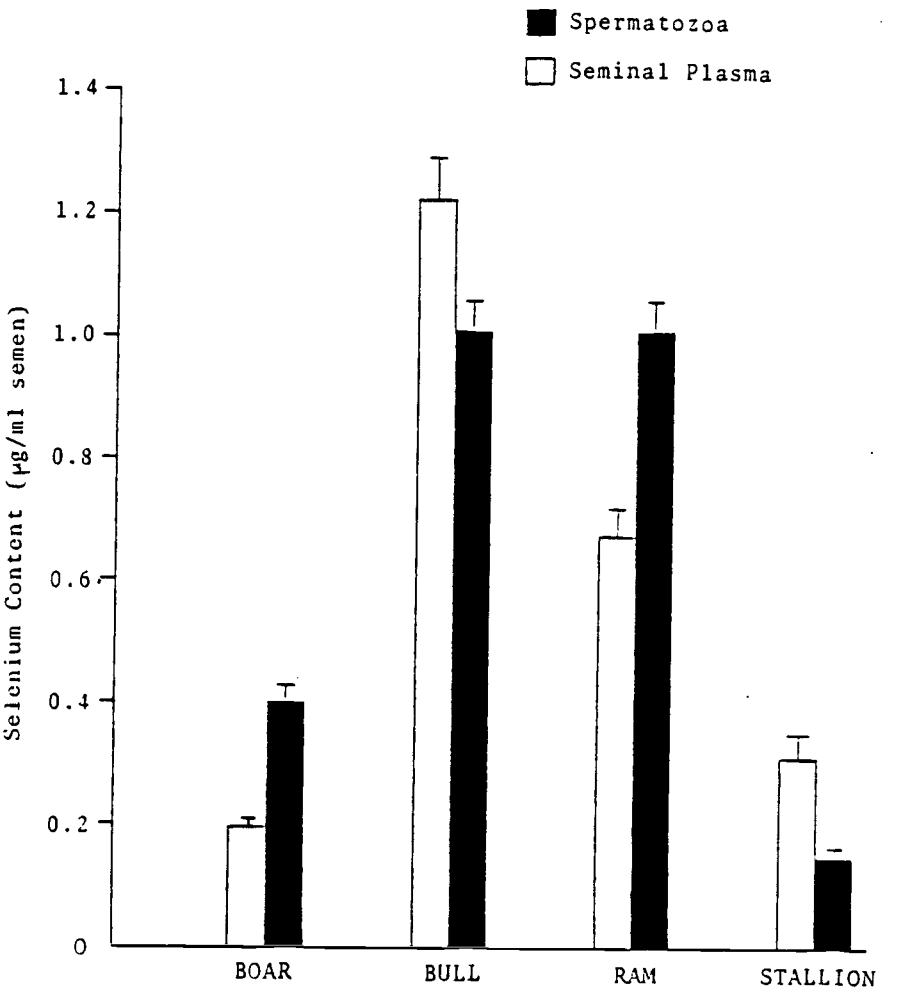


Figure 15. Selenium Distribution in Semen (Means ± SEM).

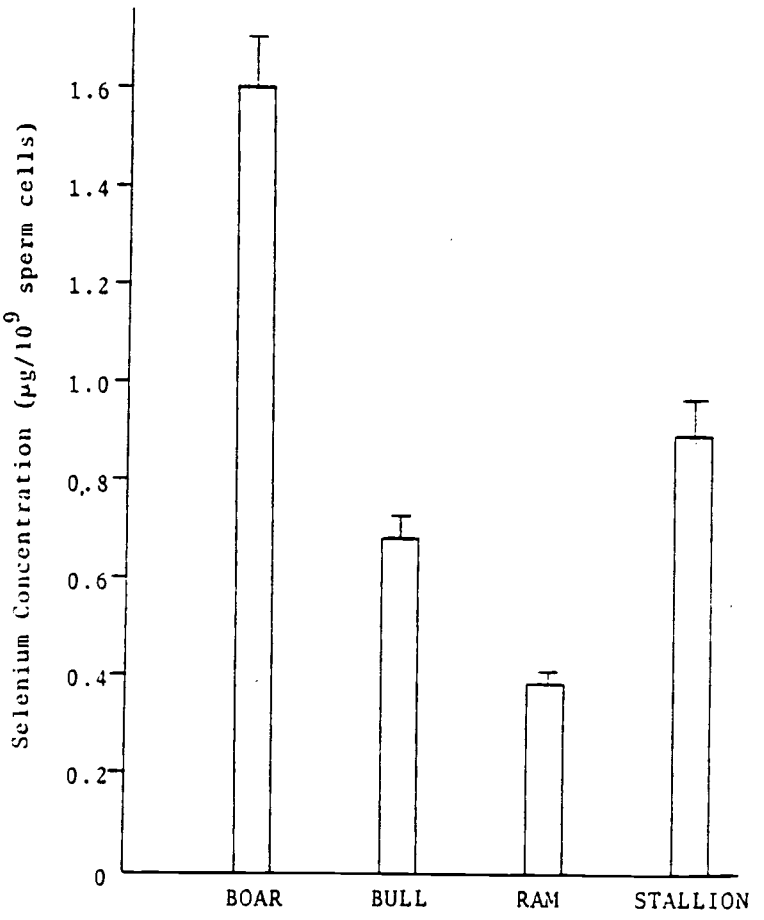


Figure 16. Selenium Levels in Spermatozoa Expressed as Micrograms per Billion Sperm Cells (Means ± SEM).

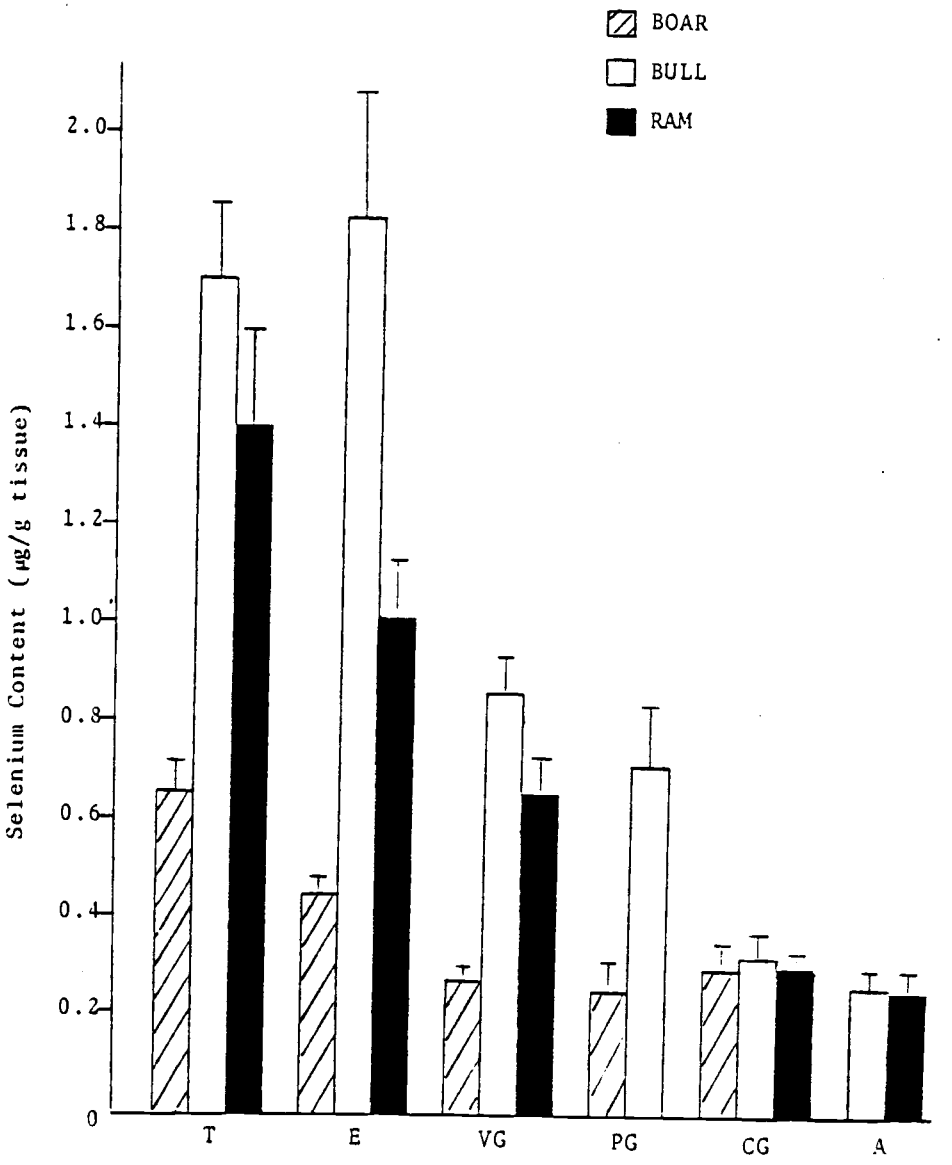


Figure 17. Selenium Distribution in Reproductive Tissues (Means ± SEM; T = Testis; E = Epipidymis; VG = Vesicular Gland; PG = Prostate Gland; CG = Cowper's Gland; A = Ampulla).

DISCUSSION

The present study has demonstrated that significant species differences exist in the distribution of GSH-Px in semen and reproductive tissues. The enzyme, GSH-Px, in the semen of bull, ram, stallion and boar was largely confined to the seminal plasma. This is in agreement with the findings of other studies with bull (Brown and Senger, 1977; Brown et al., 1977; Smith et al., 1979) and ram (Pond, 1980).

Because of the significant species differences in seminal protein content, the comparative values of GSH-Px in seminal plasma may vary according to whether the enzyme activity is expressed in terms of enzyme level per unit volume of the seminal fluid or per unit weight of protein in the fluid. GSH-Px activity determined per ml of bovine seminal plasma, for instance, was about seven times that of the ram seminal plasma. However, the activity of this enzyme in the seminal plasma of these two species appeared to be similar when GSH-Px levels were expressed in enzyme units per mg of protein because of the higher protein content found in bull seminal plasma. Since GSH-Px is an enzyme which naturally occurs in seminal plasma and is not a cellular extract, its activity in seminal plasma should be expressed in enzyme units per unit volume of seminal fluid rather than per unit weight of protein

in the fluid. It is of interest to note that in a given volume of seminal plasma, the GSH-Px level in the bull was about three times that of the stallion, seven times that of the ram and almost twenty times that of the boar. Variations of such magnitude in the levels of this enzyme in seminal plasma from different species is hard to explain. Perhaps the GSH-Px requirements of the spermatozoa of various animals are quite different. Structural damages to cell membranes, loss of phospholipids and release of lysosomal enzymes are all known to occur as a result of lipid oxidation in animal cells (Wills and Wilkinson, 1966; May and McCay, 1968). Jones and Mann (1973) reported that under aerobic conditions, ram spermatozoa produce an organic peroxide and that lipid peroxidation was probably linked with the decline in sperm respiration and motility. The presence of GSH-Px, which provides a mechanism for the removal of the harmful lipid peroxides, is therefore important for sperm function and survival.

In spite of the role of GSH-Px in cellular activity and function, the sperm cells themselves do not seem to be richly endowed with this enzyme. Although Brown and Senger (1977) reported a mean GSH-Px activity in bovine epididymal spermatozoa of 534 E.U./ml, several other investigators were unable to detect this enzyme in spermatozoa of the human (Li, 1975), bull (Brown, et al.,

1977; Smith, et al., 1977) and ram (Pond, 1980). In the present study, activity of GSH-Px was detected in the spermatozoa of bull, ram, stallion and boar, albeit in relatively small amounts. Therefore, in order to protect themselves from lipid peroxidation damage, mammalian sperm may need an endogenous GSH-Px, particularly prior to their contact with the exogenous source of GSH-Px from the accessory glands of reproduction.

Data on GSH-Px activities in reproductive tissues of the bull, ram and boar showed that vesicular tissue and its fluid contributed most of the GSH-Px found in seminal plasma. Contributions from the prostate gland were also significant but the Cowper's gland and ampulla did not contain high levels of GSH-Px. Similar findings have been reported for the ram (Pond, 1980). It has been demonstrated that GSH-Px accelerates the production of prostaglandin E (PGE) but decreases prostaglandin F (PGF) synthesis (Lands, et al., 1971; Nugteren and Hazelhof, 1973). Evidence has also been provided by Karim (1972) that PGE in human semen may assist sperm transport while PGF is antagonistic to this action. Thus, the high level of GSH-Px in vesicular glands may coincide with its well-known prostaglandin synthetic activity and the enzyme, GSH-Px, may play a significant but hitherto unrecognized role in animal reproduction via its role in regulating prostanglandin synthesis.

When the GSH-Px activity was expressed as nmoles NADPH oxidized/min/mg protein, the level of this enzyme in either testicular or epididymal tissues was much higher than that of sperm cells from the same species (Tables 2, 3, and 5). Cells in testis or epididymis other than the sperm must contribute or carry a considerable amount of GSH-Px. The exact role of GSH-Px in testis and epididymis remains to be defined. Perhaps considerable amounts of peroxides are constantly produced in these organs thus necessitating the synthesis of a peroxide-decomposing enzyme like GSH-Px in large quantities for the protection of cells from peroxidative damage.

A glutathione peroxidase, tentatively called GSH-Px II, has been found which was not active with H_2O_2 but was effective in decomposing organic hydroperoxides (Lawrence and Burk, 1976). It was suggested that this enzyme, GSH-Px II, was non selenium-dependent since it persisted in severe selenium deficiency (Prohaska and Ganther, 1976) and did not incorporate ^{75}Se *in vivo* (Prohaska and Ganther, 1977a). However, other studies (Prohaska and Ganther, 1977b; Prohaska, 1980) have provided evidence suggesting that GSH-Px II is identical to glutathione S-transferase which shows GSH-Px activity. It is interesting to note that results from this study showed no significant difference in GSH-Px response to either H_2O_2 or t-butyl O_2. The nonpreferable utilization of these two

substrates by GSH-Px clearly demonstrates that the GSH-Px in semen and reproductive tissues is predominantly of the selenium-dependent type. Lawrence and Burk (1978) reported that in rat testis GSH-Px II accounted for 91% of the total GSH-Px activity of that organ and that the livers of chick, human and sheep contained high levels of GSH-Px II. It appears then that species and tissue differences exist in the distribution of both selenium dependent GSH-Px and non selenium-dependent GSH-Px II (or, more correctly, GSH S-transferase).

Levels of selenium found in seminal plasma, vesicular gland and prostate exceeded those in blood, suggesting that the male reproductive tissues are directly involved in the uptake and accumulation of selenium. The patterns of selenium distribution in reproductive tissues of bull, ram and boar found in this study are consistent with those reported for the bull (Smith et al., 1979) and ram (Pond, 1980).

Marked species differences were observed, in this investigation, in the distribution of selenium in semen. In the boar and ram, selenium concentration was greater in spermatozoa than in seminal plasma whereas in the bull and stallion more than half of the total semen selenium content was concentrated in seminal plasma. Pond (1980) reported that, following an intramuscular injection of radioactive selenium (^{75}Se), 94% of the total semen counts

obtained from selenium-deficient ram were observed in the spermatozoa 21 days after injection. ^{75}Se counts in ejaculated spermatozoa of selenium-adequate ram increased more slowly, but accounted for more than 90% of the total semen counts 35 days post-injection. In the bull (Smith et al., 1979), however, seminal plasma counts of ^{75}Se were approximately twice the sperm counts. It appears, therefore, that the pattern of selenium distribution in semen differs from species to species. It is of interest to note that for a given number of sperm cells, the boar had the greatest concentration of selenium followed by the stallion, bull and ram in that order (Figure 16; Table 15). Further investigation is required to clarify the significance, if any, of the differences in selenium content of the spermatozoa of various species as well as the differences in the distribution of this element in semen.

In this study high levels of selenium were found in testis and epididymis, suggesting that selenium may play an important role, functional or structural, in epididymal or testicular cells. The nature of this role, however, is yet to be defined. Gunn and Gould (1970) presented evidence that selenium is incorporated by the secondary spermatocytes or early spermatids of the rat and is not directly incorporated by spermatozoa in the epididymis. Calvin (1978) reported that ^{75}Se was incorporated primar-

ily in the keratinoid proteins in the rat sperm tail, following intratesticular administration of the radioactive element. Smith et al. (1979) suggested that in the bull, selenium is incorporated in the spermatids. More recently, Pond (1980) provided evidence that in the ram, selenium is incorporated prior to or during the spermatid stage. It would appear that selenium is actively accumulated in the testis and is essential for normal spermatogenesis and for the synthesis of GSH-Px or other enzyme proteins.

Results of the present study also show a close correlation between tissue selenium content and GSH-Px activity. However, such correlation should not lead one to believe that selenium in reproductive tissues is utilized exclusively for the synthesis of GSH-Px. On the contrary, it has been shown (Martin, 1973; Ganther, 1974) that small amounts of selenium are found in most tissue proteins due to non-specific binding to protein-SH groups or due to substitution of selenoamino acids for their sulfur counterparts. In addition to this non-specific association of selenium in general, a few proteins have been isolated which contain stoichiometric quantities of selenium (Stadtman, 1974; Ganther, 1975). GSH-Px is one of the best known proteins of this type in mammals.

Research conducted by other workers has demonstrated the existence of a linear relationship between GSH-Px

activities and selenium levels in non-reproductive tissues of different species (Hafeman et al., 1974; Hoekstra, 1974; Omaye and Tappel, 1974; Oh et al., 1976). The positive relationship between erythrocyte GSH-Px activities and blood selenium levels, for example, has led to the suggestion (Chow and Tappel, 1974; Allen et al., 1975) that erythrocyte GSH-Px activity could be used as an indication of selenium status of animals. However, Whanger et al. (1977) cautioned against the indiscriminate use of erythrocyte GSH-Px levels as a criterion for determing the ram's selenium status, since the activity of GSH-Px did not correlate with blood selenium levels unless these were below the plateau concentrations.

The general decline of GSH-Px activity in seminal plasma and reproductive tissues during storage at frozen state could be attributed to the fact that GSH-Px, being a sulfhydryl-containing enzyme, is not very stable in the absence of a proper buffer and repeated freezing and thawing may enhance protein degradation. This decrease of GSH-Px resulting from freezing of seminal plasma shows the importance, for example, of extending semen with the proper substances to protect spermatozoa against the otherwise lethal effect of freezing, one of which could be the accumulation of H_2O_2 due to diminished GSH-Px activity in semen.

Fluorimetric determination of blood selenium levels

suggests that the stallions used in this investigation were probably selenium-deficient. It is therefore possible that the semen and reproductive tissue selenium and GSH-Px levels reported for the stallions are lower than would otherwise have been obtained with selenium-adequate stallions. However, it is of interest to note that, although the stallions used in this study appeared to be selenium-deficient, their spermatozoa did not show the characteristic morphological abnormalities described by Wu et al. (1969, 1971, 1973, 1979) in the spermatozoa of selenium-deficient rats. Indeed, the relatively high level of selenium found in stallion spermatozoa (0.85 $\mu g/10^9$ sperm cells) suggests that the testis of the stallion is capable of concentrating and retaining selenium and that active incorporation of selenium occurs during spermatogenesis in spite of low blood selenium levels.

SUMMARY AND CONCLUSIONS

The present study has demonstrated the presence of GSH-Px in semen and in the reproductive tissues of farm animals. It has been shown that most of the GSH-Px found in semen of the boar, bull, ram and stallion is associated with seminal plasma. Thus the importance of seminal plasma in furnishing a suitable mechanism for the removal of the otherwise toxic effects of H_2O_2 and lipid peroxides can readily be appreciated.

Results of this investigation have shown that differences or similarities could exist in GSH-Px activities between species, among individuals of the same species and between tissues depending on the units in which enzyme activity is expressed. For example, when GSH-Px activity was expressed per ml of seminal plasma, bull seminal plasma GSH-Px activity was about seven times higher than that of ram seminal plasma; however, determination of enzyme activity per mg protein showed GSH-Px activities in the two species to be similar. It has been advocated in this study that, since GSH-Px is an enzyme which naturally occurs in seminal plasma and is not a cellular extract, its activity in this fluid should be based on a unit volume of seminal plasma rather than on a unit weight of protein contained in the fluid.

Generally, GSH-Px activities were higher in bull

reproductive tissues than in those of the ram, and, in all the tissues examined, boar reproductive tissues had the least GSH-Px activities. It has been suggested that the GSH-Px requirements of the various species of farm animals differ, hence the significant differences obtained in their tissue GSH-Px levels. The relatively high activities of GSH-Px in the vesicular glands of the three species examined in this study coincide with the evidence provided by Lands et al., Nugteren and Hazelhof, 1973, among others, that this accessory gland is actively involved in the synthesis of prostaglandin E which, in humans (Karim, 1972), has been shown to assist in sperm transport.

In this study, no significant differences were observed in GSH-Px response to both H_2O_2 and t-butyl O_2 suggesting that the GSH-Px found in semen and reproductive tissues of the species used in this investigation is predominantly of the selenium-dependent type. A significant correlation was also found between tissue selenium levels and GSH-Px activities, thus providing further evidence that reproductive tissue GSH-Px of the boar, bull and ram is a selenoenzyme.

Levels of GSH-Px in seminal plasma and reproductive tissues showed a general decline with repeated non-cryoprotective freezing (at -21°C) and thawing. This demonstrates the importance of buffering tissue extracts

with appropriate chemicals before freezing for later GSH-Px assays.

In this study, it was observed that selenium levels were greater in the testis, epididymis, vesicular gland and prostate gland than in blood. This finding is consistent with the reports of other research groups (Hansson and Jacobsson, 1966; Brown and Burk, 1973; Smith et al., 1979). Relatively high amounts of selenium were also found in semen but the distribution of this element between seminal plasma and spermatozoa varied from species to species. For instance, in the bull and stallion a greater proportion of the selenium found in the semen was associated with the seminal plasma; in the boar and ram, however, the sperm contained more than half of the total semen selenium content. It has been postulated that most of the selenium in seminal plasma exists as an integral part of GSH-Px (Smith et al., 1979) whereas the selenium in spermatozoa serves primarily in a structural capacity as a component of both the keratinoid proteins of the sperm tail and the membrane system of the mitochondria (Calvin and Cooper, 1979; Pallini and Bacci, 1979; Pond, 1980).

Finally, results obtained in the present study demonstrate that GSH-Px is, to a large extent, correlated with tissue selenium levels; that the male reproductive system accumulates selenium at levels exceeding those of

blood selenium; that vesicular and prostate glands contribute significantly to the selenium and GSH-Px content of seminal plasma and that selenium, as a constituent of GSH-Px and some structural proteins of the sperm, plays a vital role in the male reproductive process, the scope of which remains to be clearly defined. Herein lies some excellent material for future experimentation.

BIBLIOGRAPHY

Allen, W. M., W. H. Parr, P. H. Anderson, S. Berrett, R. Bradley and S. S. P. Patterson. 1975. Selenium and the activity of glutathione peroxidase in bovine erythrocytes. Vet. Rec. 96:360-361.

Awasthi, Y. C., E. Beutler and S. K. Srivastave. 1975. Purification and properties of human erythrocyte glutathione peroxidase. J. Biol. Chem. 250:5144-5149.

Backstrom, S. 1958. Glutathione in aging sperm and developing eggs of the sea urchin. Arkiv. Zool. II:11:441-446.

Bieri, J. G. 1959. An effect of selenium and cystine on lipid peroxidation in tissues deficient in vitamin E. Nature 184:1143-1149.

Brown, D. G. and R. F. Burk. 1973. Selenium retention in tissues and sperm of rats fed a torula yeast diet. J. Nutr. 102:102-108.

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

Brown, D. V. and P. L. Senger. 1977. Glutathione peroxidase in bovine ejaculated semen, seminal plasma and epididymal spermatozoa. Proc. 69th Ann. Meeting Amer. Soc. Anim. Sci. Abstr. p. 141.

Brown, M. W. and J. H. Watkinson. 1977. An automated fluorimetric method for the determination of nanogram quantities of selenium. Anal. Chim. Acta. 89:29-35.

Bull, R. C. and J. E. Oldfield. 1967. Selenium involvement in the oxidation by rat liver tissue of certain tricarboxylic acid cycle intermediates. J. Nutr. 91:237-246.

Calvert, C. C., M. C. Nesheim and M. L. Scott. 1962. Effectiveness of selenium in prevention of nutritional muscular dystrophy in the chick. Proc. Soc. Exp. Biol. Med. 109:16-18.

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

Calvin, H. I. and G. W. Cooper. 1979. A specific seleno-polypeptide associated with the outer membrane of rat sperm mitochondria. In: The Spermatozoon (D. W. Fawcett and J. M. Bedford, eds.). Baltimore, Urban and Schwarzenberg, Inc. pp. 135-140.

Chow, C. K. and A. L. Tappel. 1972. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. Lipids 7:518-524.

Chow, C. K. and A. L. Tappel. 1974. Response of glutathione peroxidase to dietary selenium in rats. J. Nutr. 104:444-451.

Christopherson, B. O. 1968. Formation of monohydroxy-polyenic fatty acids from lipid peroxides by a glutathione peroxidase. Biochim. Biophys. Acta 164:35-46.

Cohen, G. and P. Hochstein. 1963. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. Biochem. 2:1420-1428.

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

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

Ganther, H. E. 1974. Biochemistry of selenium. In: Selenium (W. C. Copper and R. A. Zingaro, eds.). Van Nostrand Reinhold, New York. pp. 546-614.

Ganther, H. E. 1975. Selenoproteins. Chemica Scripta 8A:79-84.

Godwin, K. O., C. N. Fuss and R. E. Kuchel. 1975. Glutathione peroxidase activities in sheep and rat muscle and some effects of selenium deficiency. Austral. J. Biol. Sci. 28:251-258.

Gunn, S. A. and T. C. Gould. 1970. Cadmium and other mineral elements. In: The Testis. Vol. 3 (A. D. Johnson, W. R. Gomes and N. L. Vandemark, eds.). Academic Press, New York. pp. 400-404.

Hansson, E. and S-O. Jacobsson. 1966. Uptake of (^{75}Se) selenomethionine in the tissues of the mouse studied by whole-body autoradiography. Biochim. Biophys. Acta 115:285-293.

Hochstein, P. and H. Utley. 1968. Hodrygen detoxication by glutathione peroxidase and catalase in rat liver homogenates. Mol. Pharmacol. 4:574-579.

Hoekstra, W. G. 1974. Biochemical role of selenium. In: Trace Element Metabolism in Animals--2 (W. G. Hoekstra, J. W. Suttle, H. E. Ganther and W. Mertz, eds.). University Park Press, Baltimore, Maryland. pp. 61-67.

Jocelyn, P. C. 1958. Glutathione metabolixm in animals. Biochem. Soc. (Lond.) Symp. 17:43-65.

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

Karim, S. M. M. 1972. Prostaglandins and human reproduction: physiological roles and chemical uses of prostaglandins in relation to human reproduction. In: The Prostaglandins (S. M. M. Karim, ed.). New York, Wiley-Interscience.

Knox, W. E. 1960. Glutathione. In: "The Enzymes," 2nd edition, Vol. 2 (P. D. Boyer, H. Lardy and K. Myrback, eds.). Academic Press, N. Y. pp. 253-294.

Kosower, N. S., Y. Marikovsky, B. Wertheim and D. Danon. 1971. Glutathione oxidation and biophysical aspects of injury to human erythrocytes. J. Lab. Clin. Med. 78:533-545.

Lands, W., R. Lee and W. Smith. 1971. Factors regulating the biosynthesis of various prostaglandins. Ann. N. Y. Acad. Sci. 180:107-122.

Lawrence, R. A. and R. F. Burk. 1976. Non selenium-dependent glutathione peroxidase activity in rat liver. Federation Proc. 35:578.

Lawrence, R. A. and R. F. Burk. 1978. Species, tissue and subcellular distribution of non selenium-dependent glutathione peroxidase activity. J. Nutr. 108:211-215.

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

Little, C. and P. J. O'Brien. 1968. An intracellular GSH-peroxidase with a lipid peroxide substrate. Biochem. Biophys. Res. Commun. 31:145-150.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

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

MacLeod, J. 1951. Sulfhydryl groups in relation to the metabolism and motility of human spermatozoa. J. Gen Physiol. 34:705-714.

Mann, T. 1964. "The Biochemistry of Semen and of the Male Reproductive Tract." John Wiley and Sons, New York.

Martin, J. L. 1973. Selenium assimilation in animals. In: Organic Selenium Compounds: Their Chemistry and Biology (D. L. Klayman and W. H. H. Gunther, eds.). Wiley-Interscience. pp. 663-691.

May, H. E. and P. B. McCay. 1966. Reduced triphosphopridine nucleotide oxidase-catalized alternations of membrane phospholipids. 1. Nature of the lipid alternations. J. Bio. Chem. 243: 2288-2295.

Meister, A. 1973. On the enzymology of amino acid trasport. Science 180:33-39.

Mills, G. C. and H. P. Randall. 1958. Hemoglobin Catabolism II: The protection of hemoglobin from oxidative breakdown in the intact erythrocyte. J. Biol. Chem. 232-589-598.

Neubert, D., A. B. Wojtczalc and A. L. Lehninger. 1962. Purification and enzymatic identity of mitochondrial contraction-factors I and II. Proc. Nat. Acad. Sci. 48:1651-1658.

Niemi, S. M., F. B. Kuzan and P. L. Senger. 1981. Selenium in bovine spermatozoa. J. Dairy Sci. 64:853-856.

Noguchi, T., A. H. Cantor and M. L. Scott. 1973. Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. J. Nutr. 103:1502-1511.

Nugteren, D. H. and E. Hazelhof. 1973. Isolation and properties of intermediates in prostaglandin biosynthesis. Biochim. Biophys. Acta: 326: 448-461.

O'Brien, P. J. and A. C. Frazer. 1966. The effect of lipid peroxides on the biochemical constituents of the cell. Proc. Nutr. Soc. 25:9-18.

Oh, S. H., H. E. Ganther and W. G. Hoekstra. 1974. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. Biochem. 13:1825-1829.

Omaye, S. T. and A. L. Tappel. 1974. Effect of dietary selenium on glutathione peroxidase in the chick. J. Nutr. 104:747-753.

Paglia, E. E. and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

Pallini, V. and E. Bacci. 1979. Bull sperm selenium is bound to a structural protein of mitochondria. J. Submicro. Cytol. 11:168-170.

Pond, F. R. 1980. Selenium-75 incorporation in semen and reproductive organs of the bull and ram. Ph.D. thesis, Oregon State University, Corvallis, Oregon. 81 numb. leaves.

Prohaska, J. R. and H. E. Ganther. 1976. Selenium and glutathione peroxidase in developing rat brain. J. Neurochem. 27:1379-1387.

Prohaska, J. R. and H. E. Ganther. 1977a. Association of GSH:organic hydroperoxide oxidoreductase activity with GSH s-transferase A activity in testicular cytosol. Fed. Proc., Fed. Amer. Soc. Exp. Biol. 36:4349 (abstr.).

Prohaska, J. R. and H. E. Ganther. 1977b. Glutathione peroxidase activity of GSH s-transferase purified from rat liver. Biochem. and Biophys. Res. Commun. 76:437-445.

Prohaska, J. R. 1980. The glutathione peroxidase activity of glutathione s-transferase. Biochim. Biophys. Acta 611:87-98.

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

Schwartz, K. and C. M. Foltz. 1957. Selenium as an integral part of α-Factor 3 against dietary necrotic liver degeneration. J. Amer. Chem. Soc. 79:3292-3293.

Scott, M. L. 1966. Studies on the inter-relationship of selenium, vitamin E and sulfur amino acids in a nutritional myopathy of the chick. Ann. N. Y. Acad. Sci. 138:82-89.

Smith, P. J., A. L. Tappel and C. K. Chow. 1974. Glutathione peroxidase activity as a function of dietary selenomethionine. Nature 247:392-393.

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

Stadtman, T. C. 1974. Selenium biochemistry: proteins containing selenium are essential components of certain bacterial and mammalian enzyme systems. Science 183:915-922.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill Book Co., Inc. New York.

Tosic, J. and A. Walton. 1950. Metabolism of spermatozoa. The formation and elimination of hydrogen peroxide by spermatozoa and effects on motility and survival. Biochem. J. 47:199-212.

VanDemark, N. L., G. W. Salisbury and R. W. Bratton. 1949. Oxygen damage to bull spermatozoa and its prevention by catalase. J. Dairy Sci. 32:353-360.

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

Whanger, P. D., N. D. Pederson and P. H. Weswig. 1973. Selenium proteins in ovine tissues, II: Spectral properties of 10,000 molecular weight selenium protein. Biochem. Biophys. Res. Commun. 53:1031-1035.

Whanger, P. D., P. H. Weswig, J. A. Schmitz and J. E. Oldfield. 1977. Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. J. Nutr. 107:1298-1307.

White, I. G. 1955. The toxicity of heavy metals to mammalian spermatozoa. Austral. J. Exp. Biol. 33:359-366.

Wills, E. D. and A. E. Wilkinson. 1966. Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. Biochem. J. 99:657-666.

Wu, E. H., J. E. Oldfield, O. H. Muth, P. D. Whanger and P. H. Weswig. 1969. Effect of selenium on reproduction. Proc. West. Sec., Amer. Soc. An. Sci. 20:85-89.

Wu, S. H., J. E. Oldfield and P. D. Whanger. 1971. Effect of selenium, chromium and vitamin E on spermatogenesis. J. Anim. Sci. 33:273 (abstr.).

Wu, S. H., J. E. Oldfield, P. D. Whanger and P. H. Weswig. 1973. Effect of selenium, vitamin E and anitoxidants on testicular function in rats. Biol. Reprod. 8:625-629.

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

A P P E N D I X

Tables 1 - 12 show the GSH-Px activities in semen and reproductive tissues of farm animals (E.U. = Enzyme Units = nanomoles NADPH oxidized/min). Means ± SEM within a column not sharing a common superscript letter or numeral are significantly different at levels indicated for each table. All statistical comparisons were made using factorial analysis of variance (Steel and Torrie, 1980).

Table 1. Glutathione Peroxidase Activity in Seminal Plasma

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	8.8 ± 0.4^a	8.6 ± 0.4^a	184 ± 23^1	176 ± 19^1
Bull	45.6 ± 5.4^b	44.6 ± 6.6^b	3523 ± 643^2	3480 ± 680^2
Ram	42.0 ± 8.1^b	41.3 ± 7.8^b	520 ± 45^3	508 ± 39^3
Stallion	21.3 ± 1.4^c	20.9 ± 1.2^c	1250 ± 73^4	1125 ± 51^4

a and b, 1 and 2, 1 and 4, or 1 and 3: $P < 0.005$
a and c, b and c, 2 and 4, or 3 and 4: $P < 0.01$

Table 2. Glutathione Peroxidase Activity in Spermatozoa

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml semen	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	0.19 ± 0.02^a	0.18 ± 0.03^a	25 ± 3^1	24 ± 4^1
Bull	1.00 ± 0.13^b	0.98 ± 0.13^b	127 ± 18^2	125 ± 16^2
Ram	1.40 ± 0.17^b	1.36 ± 0.18^b	193 ± 10^3	189 ± 12^3
Stallion	0.36 ± 0.02^a	0.34 ± 0.02^a	38 ± 1^1	36 ± 2^1

a and b or 2 and 3: $P < 0.05$; 1 and 2 or 1 and 3: $P < 0.01$

Table 3. Glutathione Peroxidase Activity in Spermatozoa Expressed as E.U./10^9 Sperm Cells

Species	t-butyl O_2	H_2O_2
Boar	100.0 ± 16[a]	96.0 ± 15[a]
Bull	84.7 ± 13[a]	83.3 ± 15[a]
Ram	77.2 ± 11[a]	75.6 ± 9[a]
Stallion	190.0 ± 22[b]	180.0 ± 19[b]

a and b: $P < 0.01$

Table 4. Glutathione Peroxidase Activity in Testicular Parenchyma

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	17.4 ± 0.7^a	16.4 ± 0.3^a	165 ± 40^1	156 ± 41^1
Bull	59.3 ± 1.7^b	58.5 ± 2.1^b	610 ± 26^2	600 ± 27^2
Ram	59.6 ± 5.1^b	58.4 ± 4.5^b	544 ± 57^3	535 ± 54^3

a and b or 1 and 2: $P < 0.005$; 2 and 3: $P < 0.05$

Table 5. Glutathione Peroxidase Activity in Testicular Fluid

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	9.5 ± 0.8^a	9.2 ± 0.6^a	106 ± 21^1	92 ± 18^1
Bull	28.1 ± 1.6^b	26.7 ± 1.4^b	295 ± 18^2	280 ± 19^2
Ram	28.2 ± 1.4^b	26.5 ± 1.2^b	285 ± 18^2	268 ± 15^2

a and b or 1 and 2: $P < 0.01$

Table 6. Glutathione Peroxidase Activity in Epididymal Tissue

	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
Species	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	10.2 ± 0.3^a	9.4 ± 0.6^a	131 ± 26^1	120 ± 28^1
Bull	21.2 ± 1.5^b	20.4 ± 1.6^b	339 ± 44^2	325 ± 42^2
Ram	15.7 ± 1.6^c	15.3 ± 1.6^c	240 ± 36^3	206 ± 32^3

a and b or 1 and 2: $P < 0.01$; b and c or 2 and 3: $P < 0.05$

Table 7. Glutathione Peroxidase Activity in Epididymal Fluid

	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
Species	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	3.3 ± 0.5	3.4 ± 0.8	65 ± 13^1	67 ± 14^1
Bull	5.0 ± 0.5	4.9 ± 0.4	173 ± 9^2	162 ± 7^2
Ram	5.7 ± 0.3	5.4 ± 0.4	108 ± 11^3	103 ± 12^3

1 and 2: $P < 0.01$; 2 and 3: $P < 0.05$

Table 8. Glutathione Peroxidase Activity in Vesicular Tissue

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	1.9 ± 0.5^a	2.0 ± 0.6^a	105 ± 28^1	112 ± 24^1
Bull	3.3 ± 0.3^a	3.1 ± 0.3^b	235 ± 21^2	223 ± 23^2
Ram	6.9 ± 0.5^b	6.8 ± 0.4^b	440 ± 33^3	431 ± 29^3

a and b or 1 and 2: $P < 0.05$; 1 and 3: $P < 0.005$; 2 and 3: $P < 0.01$

Table 9. Glutathione Peroxidase Activity in Vesicular Fluid

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	2.4 ± 1.3^a	2.2 ± 0.9^a	198 ± 21^1	185 ± 19^1
Bull	15.7 ± 0.9^b	15.6 ± 1.0^b	1525 ± 47^2	1500 ± 56^2
Ram	10.7 ± 0.4^c	10.6 ± 0.4^c	909 ± 36^3	902 ± 34^3

a and b or 2 and 3: $P < 0.01$; b and c: $P < 0.05$; 1 and 2: $P < 0.005$

Table 10. Glutathione Peroxidase Activity in Prostate Gland

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	2.6 ± 0.4^a	2.4 ± 0.3^a	134 ± 38^1	122 ± 32^1
Bull	12.0 ± 0.7^b	11.7 ± 0.6^b	548 ± 29^2	526 ± 27^2

a and b or 1 and 2: $P < 0.01$

Table 11. Glutathione Peroxidase Activity in Cowper's Gland

Species	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Boar	1.3 ± 0.1^a	1.2 ± 0.2^a	107 ± 20^1	101 ± 23^1
Bull	3.5 ± 0.5^b	3.3 ± 0.5^b	165 ± 28^2	154 ± 25^2
Ram	6.9 ± 0.5^c	6.7 ± 0.4^c	220 ± 13^3	200 ± 11^3

a and b, b and c, 1 and 2, or 2 and 3: $P < 0.05$;
a and c or 1 and 3: $P < 0.01$

Table 12. Glutathione Peroxidase Activity in Ampulla

	Enzyme activity E.U./mg protein		Enzyme activity E.U./ml	
Species	t-butyl O_2	H_2O_2	t-butyl O_2	H_2O_2
Bull	2.2 ± 0.4	2.1 ± 0.3	188 ± 31[1]	175 ± 28[1]
Ram	1.4 ± 0.1	1.4 ± 0.1	95 ± 4[2]	87 ± 4[2]

1 and 2: $P < 0.01$

Table 13. Effect of Repeated Freeze-Thawing on GSH-Px Activity

Tissue/ Fluid	Species	DAYS OF FREEZING AT -21°C										
		0	5	10	15	20	25	30	35	40	45	50
Seminal plasma	Boar	8.6	9.1	9.6	10.0	9.4	8.8	8.2	7.6	7.5	7.1	7.0
	Bull	44.6	42.5	34.6	38.0	38.0	30.0	25.5	24.8	24.6	23.8	20.6
	Ram	41.3	45.6	40.7	40.1	32.0	25.2	24.3	23.1	20.1	16.5	10.7
	Stallion	20.9	20.6	17.8	16.2	14.5	10.4	7.4	6.3	5.9	5.6	5.4
Testicular Parenchyma	Boar	16.4	16.4	16.3	16.0	15.8	15.8	15.1	14.4	14.2	13.7	13.5
	Bull	58.5	52.5	51.5	45.0	43.0	40.2	36.1	35.0	34.2	32.0	30.5
	Ram	58.4	56.3	50.8	42.1	37.5	33.0	31.2	28.5	26.0	24.3	22.5
Testicular fluid	Boar	9.2	9.2	9.0	8.7	8.8	8.5	8.5	8.2	8.1	8.0	8.0
	Bull	26.7	26.6	26.6	26.4	26.8	26.5	25.2	24.6	24.3	24.1	23.5
	Ram	26.5	26.2	25.8	25.4	23.7	23.0	22.8	22.3	20.1	19.4	18.2
Epididymal tissue	Boar	9.4	9.2	9.2	9.0	9.0	9.0	8.7	8.6	8.6	8.6	8.6
	Bull	20.4	21.3	19.8	19.6	19.5	19.1	18.6	18.0	16.5	14.0	13.8
	Ram	15.3	15.1	15.1	15.1	15.3	15.0	15.0	15.0	14.6	14.3	14.0
Epididymal fluid	Boar	3.4	3.4	3.4	3.4	3.4	3.2	3.2	3.0	3.5	3.3	3.3
	Bull	4.9	5.0	4.7	4.8	4.5	4.3	4.2	3.6	3.2	2.9	2.8
	Ram	5.4	5.3	5.6	5.1	4.7	4.3	3.8	3.4	3.0	2.7	2.5
Vesicular tissue	Boar	2.0	2.0	2.0	2.1	2.0	1.9	1.9	1.8	1.9	1.6	1.6
	Bull	3.1	3.1	3.1	3.0	2.8	3.0	3.0	3.1	3.0	2.8	2.8
	Ram	6.8	6.8	6.5	6.2	6.1	5.9	5.8	5.5	5.2	5.0	4.7
Vesicular fluid	Boar	2.2	2.1	2.0	2.0	2.1	2.1	1.8	1.7	1.7	1.7	1.7
	Bull	15.6	15.2	15.0	14.7	14.7	14.5	13.4	12.6	12.0	10.2	9.5
	Ram	10.6	10.5	10.3	9.8	9.3	8.6	8.4	8.1	7.8	7.6	7.5
Prostate	Boar	2.4	2.4	2.4	2.3	2.3	2.0	2.0	1.8	1.6	1.6	1.5
	Bull	11.7	11.5	11.1	11.2	11.3	10.6	9.5	9.0	8.8	8.8	8.7
Cowper's gland	Boar	1.2	1.0	1.0	0.8	0.8	0.8	0.6	0.5	0.5	0.4	0.2
	Bull	3.3	3.2	3.1	3.0	3.0	3.1	3.0	2.9	2.0	1.6	1.6
	Ram	6.7	6.6	6.7	6.8	6.8	6.5	6.3	6.0	5.9	5.7	5.5
Ampulla	Bull	2.1	2.1	1.9	1.7	1.7	1.6	1.5	1.3	1.0	0.8	0.8
	Ram	1.4	1.4	1.5	1.2	1.2	1.0	0.9	0.9	0.6	0.5	0.5

*Obtained with H_2O_2 as substrate. Values expressed as E.U./mg protein.

Table 14. Selenium (Se) Distribution in Semen

	Seminal Plasma		Spermatozoa	
	Se content µg/ml semen	% of semen Se content	Se content µg/ml semen	% of semen Se content
Boar	0.22 ± 0.01[a]	35.7	0.40 ± 0.02[a]	64.3
Bull	1.24 ± 0.08[b]	55.3	1.01 ± 0.06[b]	44.7
Ram	0.66 ± 0.05[a]	39.4	1.01 ± 0.06[b]	60.6
Stallion	0.32 ± 0.03[a]	65.5	0.17 ± 0.01[a]	34.5

Means (± SEM) followed by different superscripts are significantly ($P < 0.05$) different.

Table 15. Selenium Concentration in Spermatozoa (µg/10^9 sperm cells)

Boar	Bull	Ram	Stallion
1.6 ± 0.09[a]	0.67 ± 0.04[b]	0.41 ± 0.02[b]	0.85 ± 0.06[b]

Means (±SEM) followed by different superscripts are significantly ($P < 0.05$) different.

Table 16. Selenium[1] Distribution in Reproductive Tissues

Tissue	Boar	Bull	Ram
Testis	0.65 ± 0.06[a]	1.70 ± 0.14[b]	1.40 ± 0.20[b]
Epididymis	0.45 ± 0.04[a]	1.83 ± 0.26[b]	1.04 ± 0.12[b]
Vesicular Gland	0.28 ± 0.03[c]	0.85 ± 0.10[a]	0.65 ± 0.10[a]
Prostate Gland	0.26 ± 0.03[c]	0.72 ± 0.11[a]	———
Cowper's Gland	0.30 ± 0.05[c]	0.34 ± 0.05[c]	0.31 ± 0.02[c]
Ampulla	———	0.27 ± 0.04[c]	0.25 ± 0.05[c]

[1]Selenium content expressed as µg/g tissue.

Means (± SEM) followed by different superscripts are significantly ($P < 0.05$) different.