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Influence of Injected Selenium in Dairy Bulls on Blood and Semen Selenium, Glutathione Peroxidase and Seminal Quality[1,2]

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ABSTRACT

A 9 month study was conducted to determine the influence of selenium (Se) injections on blood and semen Se and glutathione peroxidase (GSH-Px) levels in bulls that were moderately Se-deficient. The effects of Se injections on semen production and quality were also examined. Five Holstein bulls were assigned to either a Se or no Se treatment based on their pretreatment seminal production and quality characteristics. Three bulls were given i.m. injections of 5, 10, 20, or 40 mg Se as sodium selenite per 90 kg BW at 6, 16, 22, and 28 weeks, respectively, after initiation of the experiment. Two bulls received sham injections at the same times and served as controls. Blood samples were collected weekly from each bull and assayed for GSH-Px and selenium. Semen was collected 3 times weekly from each bull and assayed for GSH-Px activity in the seminal plasma. Thirteen weeks after initiation of the experiment, semen from each bull was analyzed for Se on a weekly basis. Ejaculates from each bull were evaluated for volume, concentration of spermatozoa, percentage of motile spermatozoa, percentage of intact acrosomes and spermatozoal morphology immediately postcollection. Post-thaw semen quality was determined from two ejaculates from each bull per week. Frozen semen was thawed, incubated for 4 h at 37°C, and spermatozoa were evaluated for post-thaw percentage of motile spermatozoa and percentage of intact acrosomes.

Selenium injections increased blood Se ($P<0.05$), blood GSH-Px ($P<0.005$), semen Se ($P<0.06$), and seminal plasma GSH-Px ($P<0.05$). Blood Se and GSH-Px did not increase until after the 10 mg injection. Seminal plasma GSH-Px appeared highly sensitive to changes in Se status since enzyme levels tripled within 48 h following the 5 mg injection. Subsequent increases in seminal plasma GSH-Px were observed with each Se injection.

Neither postcollection nor post-thaw semen quality was influenced by Se injections.

INTRODUCTION

Recent studies have shown that Se is associated with the male reproductive system. Studies using ^{75}Se showed marked uptake of Se by the testis in rats (Brown and Burk, 1972), mice (Gunn et al., 1967), and bulls (Smith et al., 1979). Following a single injection of ^{75}Se, most tissues acquired and then lost the isotope quickly while the testis continued to accumulate it (Brown and Burk, 1972; Gunn et al., 1967). Brown and Burk (1972) reported that 40% of whole-body ^{75}Se in the Se-deficient rat was found in the testis 3 weeks after a single [^{75}Se]-selenite injection. After 3 weeks, the concentration of ^{75}Se declined in the testis but increased in the epididymis suggesting that Se was incorporated into spermatozoa in the testis. These results are supported by the work of Smith et al. (1979) who demonstrated that, in the bull, ^{75}Se retention in the epididymis was highly correlated ($r = 0.92$, $P<0.01$) with spermatozoal concentration. Smith et al. (1979) also demonstrated that when ejaculated bovine spermatozoa were subjected to repeated freezing and thawing in distilled water, the Se remained with the spermatozoa, suggesting that Se was tightly bound to the structural components of the cell. Calvin (1978) has shown that the flagellum of rat spermatozoa contain a specific selenopolypeptide which he termed "selenoflagellin." He suggested that this

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protein is important in the formation and function of the flagellum.

In addition to its association with spermatozoal protein, Se is also an essential component of the enzyme glutathione peroxidase (GSH-Px) (Rotruck et al., 1973). This enzyme is found in most mammalian tissues where it protects cell membranes against oxidative damage (Mills and Randall, 1958). Glutathione peroxidase has also been found in the semen of rams, dogs, humans, goats (Li, 1975), and bulls (Brown et al., 1977; Smith et al., 1979). However, the physiologic function of this selenoenzyme in semen has yet to be determined.

In spite of the fact that Se has been shown to be associated with the male reproductive system, no information is available regarding the levels of blood and semen Se and GSH-Px in bulls. Furthermore, the influence of Se injections on semen quality in reproductively active bulls has not been described. Therefore, the primary objective of this study was to describe the changes in blood and semen Se and GSH-Px activity following Se injections in bulls that were determined to have low blood Se levels (<0.05 ppm Se). A second objective was to determine the influence of Se injections on semen production and spermatozoal quality.

MATERIALS AND METHODS

The experiment was divided into a pre- and post-treatment period. Bulls were determined to be of marginal Se status prior to the initiation of the study. In the pretreatment period, semen was collected from five Holstein bulls (∿19 months of age) 3 times weekly for 5 weeks. Bulls were ranked according to seminal production and quality so that they could be assigned to either the Se or no Se treatment. Semen quality of all bulls was within acceptable limits at the onset of the experiment. Since semen production and quality may vary independently among bulls, and since it was not known which variables (if any) would be affected by Se, an index which incorporated several variables was used to rank the bulls. The index was generated using the following equation:

$$\text{Index} = \text{PIA} \times \%\ \text{Normal Sperm} \times \text{Total Sperm}$$

where PIA is percentage of intact acrosomes (mean of post-thaw evaluations at 0, 4, and 8 h), % Normal Sperm the percent normal spermatozoa as determined by morphological evaluation, and Total Sperm the total number of spermatozoa per ejaculate. An index value for each bull was computed using the overall mean of each variable from the pretreatment period. The bull with the highest index value arbitrarily received the Se treatment. The second- and third-ranked bulls were randomly assigned to either the Se or no Se treatment, as were the fourth- and fifth-ranked bulls. Following the pretreatment period, the three bulls assigned to the Se treatment were injected i.m. with 5 mg sodium selenite per 90 kg BW. Polysorbate was the injection vehicle and control bulls were given injections of polysorbate only. Because the first injection did not elicit a blood Se response within 10 weeks, subsequent injections of 10, 20, and 40 mg sodium selenite per 90 kg BW were administered at 10, 16, and 22 weeks following the initial injection to maintain high blood Se levels.

Semen was collected by artificial vagina throughout the experiment. The collection regimen for all bulls included the following: one false-mount followed by 2 min restraint; a second false-mount followed by 2 min restraint and collection (Almquist, 1978). This regimen was maintained throughout the experiment. Frequency of collection was three ejaculates per week for the first 15 weeks of the experiment, four ejaculates per week for the next 15 weeks, and six ejaculates per week for the duration of the experiment. On days when more than one ejaculate was collected, ejaculates were pooled and considered as a single ejaculate. The purpose of increasing the frequency of collection was to determine whether Se injections could affect semen production and quality in bulls subjected to increased sexual activity. Seminal volume and concentration of spermatozoa were determined for all ejaculates immediately after collection. An aliquot (2 ml) of neat semen was then removed, packaged in 0.5 ml French straws, frozen and stored in liquid nitrogen (LN) for subsequent GSH-Px assay. Postcollection motility was estimated using phase contrast microscopy (160 X) from semen diluted in egg yolk-citrate immediately after collection. Immediately following motility estimates, semen samples were fixed in glutaraldehyde (0.05 ml 1% GLUT/ml diluted semen) by a modification of the procedure of Johnson et al. (1976) and evaluated within 2 to 4 h postcollection for intact acrosomes (Saacke and White, 1972) and spermatozoal morphology, using differential interference contrast microscopy (1000 X). Spermatozoa were not considered normal if they had an abnormal head or tail, cytoplasmic droplets, or nuclear vacuoles.

To determine the effect of Se on the ability of spermatozoa to withstand the freeze-thaw process, 1 ml of the remaining neat semen was removed from each of two ejaculates per week from each bull and diluted in egg yolk-citrate-glycerol. Initial dilution was made in 10 ml of egg yolk-citrate without gylcerol (fraction "A") at 37°C. The partially diluted semen was suspended in beakers containing 200 ml of water at 37°C and cooled at 10°C (∿1.5 h). After reaching 10°C, samples were removed from the water bath, and semen was further cooled to 4°C. Following the cooling period, the remaining fraction "A" was added to give a concentration of 60×10^6 spermatozoa/ml. To complete the dilution, an equal volume of egg yolk-citrate containing 14% glycerol (fraction "B") was added to fraction "A" in 10, 20, 30, and 40% increments by volume at 10 min intervals to give a final concentration of 30×10^6 spermatozoa/ml. Semen was packaged in 0.25 ml Continental straws and allowed to equilibrate for 2 h. After equilibration, straws were frozen in static LN vapor for 10 min and stored in LN until the time of post-thaw evaluation.

Blood samples were collected weekly from each bull and assayed for Se and GSH-Px activity. Thirteen

weeks after initiation of the experiment, an aliquot of neat semen was removed from one ejaculate per bull per week for Se analysis. Selenium was assayed using the fluorometric procedure of Olson (1969). Whole blood was used for GSH-Px assay and samples were prepared by adding 1 part distilled water to 1 part blood, freezing and thawing 3 times, then adding an equal volume of Drabkin's reagent. A final dilution of 1:204 was made in 0.02 M phosphate buffer. For assay of seminal plasma GSH-Px, the previously frozen neat semen was thawed in air for 5 min and centrifuged at 30,000 × g for 10 min to separate the seminal plasma from spermatozoa. A final dilution of 1:50 was made in 0.02 M phosphate buffer. Glutathione peroxidase in both blood and seminal plasma was assayed by a procedure modified from Paglia and Valentine (1967) as described by Smith et al. (1979).

For evaluation of post-thaw seminal quality, four straws were thawed for 1 min at 37°C water. Semen from the four straws was pooled into a 12 × 75 mm test tube and immediately placed into a dry block maintained at 37°C. After 4 h of incubation, unfixed smears were evaluated for percentage of motile spermatozoa and percentage of intact acrosomes using phase contrast and differential interference contrast microscopy, respectively. Samples were coded to ensure that the evaluator was not aware of the bulls being evaluated.

Bulls were fed ∿1.4 kg of grain and 9.1 kg of alfalfa hay daily for the first 5 months of the experiment and 9.1 kg of hay daily for the remainder of the experiment. Selenium concentration was 0.07 ppm in the grain and 0.10 ppm in the hay.

The data were analyzed by analysis of variance using the following model:

$$Y_{ijk} = u + T_i + W_j + B_{ik} + e_{ijk}$$

where Y_{ijk} is the observed postcollection PIA or motility, post-thaw PIA or motility, % Normal, weekly sperm production, index value, blood GSH-Px, seminal plasma GSH-Px, semen Se or blood Se; T_i is the effect of i^{th} treatment (Se or sham injection); W_j is the effect of j^{th} time (weeks); B_{ik} is the effect of k^{th} bull within the i^{th} treatment; and e_{ijk} is random error.

RESULTS

As expected, blood Se levels increased following injections of selenium ($P<0.05$); however, the increase was not observed until after the Se dose was increased to 10 mg Se/90 kg BW (Fig. 1). Thereafter, blood Se levels in the treated bulls remained ∿4 times the amount in the untreated bulls. At the termination of the experiment, blood Se in the treated bulls was ∿0.15 ppm. About 12 weeks after the initiation of the experiment, blood Se levels in the untreated bulls decreased unexpectedly to below 0.02 ppm; however, there was a subsequent increase during the remainder of the experiment. There is no apparent explanation for this decline and subsequent rise.

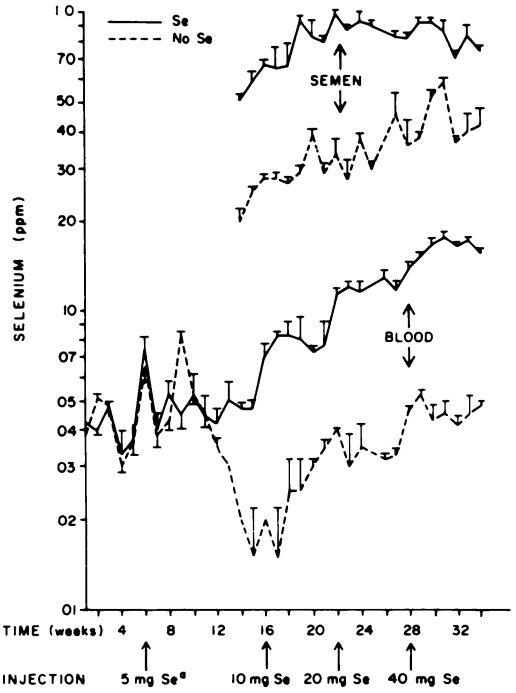

FIG. 1. Influence of Se injections on blood and semen Se levels. Each point for Se treatment represents the mean ± SEM for three bulls. Each point for no Se treatment represents the mean ± SEM for two bulls. [a]All injections were as Na selenite per 90 kg BW.

Selenium in neat semen was not monitored until 13 weeks after initiation of the experiment. When monitoring was initiated, semen Se was higher ($P<0.06$) in the treated bulls than the untreated bulls and remained higher throughout the experiment (Fig. 1). Selenium was ∿10 times higher in the semen than the blood in both treated and untreated bulls, respectively (Fig. 1).

The influence of Se injections on blood GSH-Px was similar to that observed for blood Se (Fig. 2). Although Se injections increased blood GSH-Px ($P<0.005$), the increase was not observed until ∿2 weeks after bulls received the 10 mg injection. Blood GSH-Px activity continued to increase with each Se injection with no indication of plateauing at the termination of the experiment.

Seminal plasma GSH-Px responded differently than blood GSH-Px following the Se injections. Enzyme levels in the seminal plasma increased ($P<0.05$) within 48 h after the 5 mg injection and activity almost tripled during this time (Fig. 3). After reaching a peak at about 2

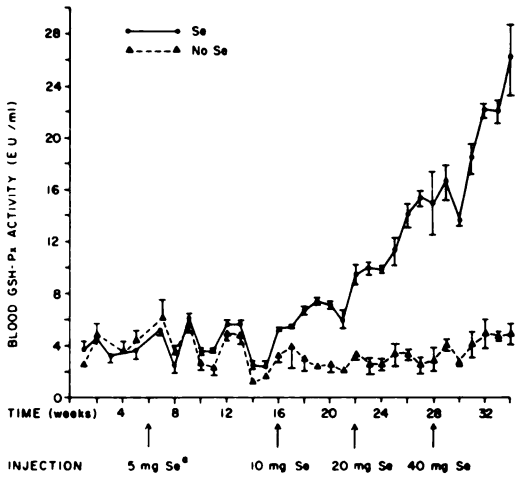

FIG. 2. Influence of Se injections on blood GSH-Px activity. Each point for Se treatment represents the mean ± SEM for three bulls. Each point for no Se treatment represents the mean ± SEM for two bulls. [a]All injections were as Na selenite per 90 kg BW.

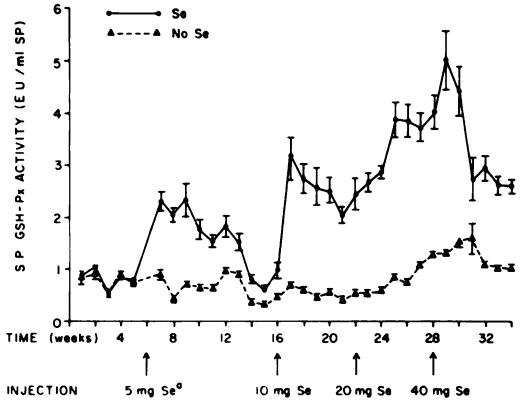

FIG. 3. Influence of Se injections on seminal plasma (S.P.) GSH-Px activity. Each point for Se treatment represents the mean ± SEM for three bulls. Each point for no Se treatment represents the mean ± SEM for two bulls. [a]All injections were as Na selenite per 90 kg BW.

weeks after the first injection (5 mg), GSH-Px activity in the treated bulls decreased to that of the untreated bulls by 9 weeks postinjection. Subsequent Se injections increased GSH-Px activity in the seminal plasma with each increase in Se dose in a manner similar to, but greater in magnitude than the first injection.

Selenium injections did not significantly improve the semen production or the semen quality variables examined (Fig. 4). Likewise Se injections did not influence the index value or weekly sperm production for the Se-treated bulls. There were no significant correlations between levels of semen Se or seminal plasma GSH-Px and semen quality.

DISCUSSION

It has been shown in several species that Se supplementation significantly increases blood Se levels (Hoffman et al., 1978; Preston and Moxon, 1972; Oh et al., 1976; Chow and Tappel, 1974). In this study, blood Se was elevated following Se injections; however, the increase was not observed until after the Se dose had been increased to 10 mg sodium selenite/90 kg BW. The lack of a blood Se response following the 5 mg injection was unexpected. In steers, Se injections as low as 2.48 mg Se as sodium selenite/90 kg BW[4] significantly increased blood Se levels from 0.05 to 0.07 ppm within 8 days after injection (Preston and Moxon, 1972), and 4.5 mg sodium selenate/90 kg BW[4] increased blood Se from 0.03 to 0.07 ppm within 2 days in lactating Holstein cows (Little et al., 1979). The present study suggests that the amount of Se required to elicit a blood Se response is higher for reproductively active bulls than for either cows or steers.

Although whole semen Se was not monitored until 13 weeks after initiation of the experiment, semen from Se-treated bulls was higher in Se ($P<0.06$) than was semen from untreated bulls throughout the sampling period. Semen Se in the treated bulls was 2.5 times higher than in the untreated bulls at the start of the sampling period. Since sampling was initiated after the 5 mg injection but before the 10 mg injection, it would appear that the 5 mg injection of Se was sufficient to elicit a Se response in the semen but not in the blood. Semen Se levels remained ∿10 times higher than blood levels throughout the experiment for both the treated and untreated bulls. It cannot be determined from this study what accounted for the significant increase in semen selenium. Since Se is associated with spermatozoa (Calvin, 1978; Smith et al., 1979) and spermatozoal production did not increase ($P>0.2$), the increase in semen Se was not due

[4] Values were converted to Se/90 kg BW.

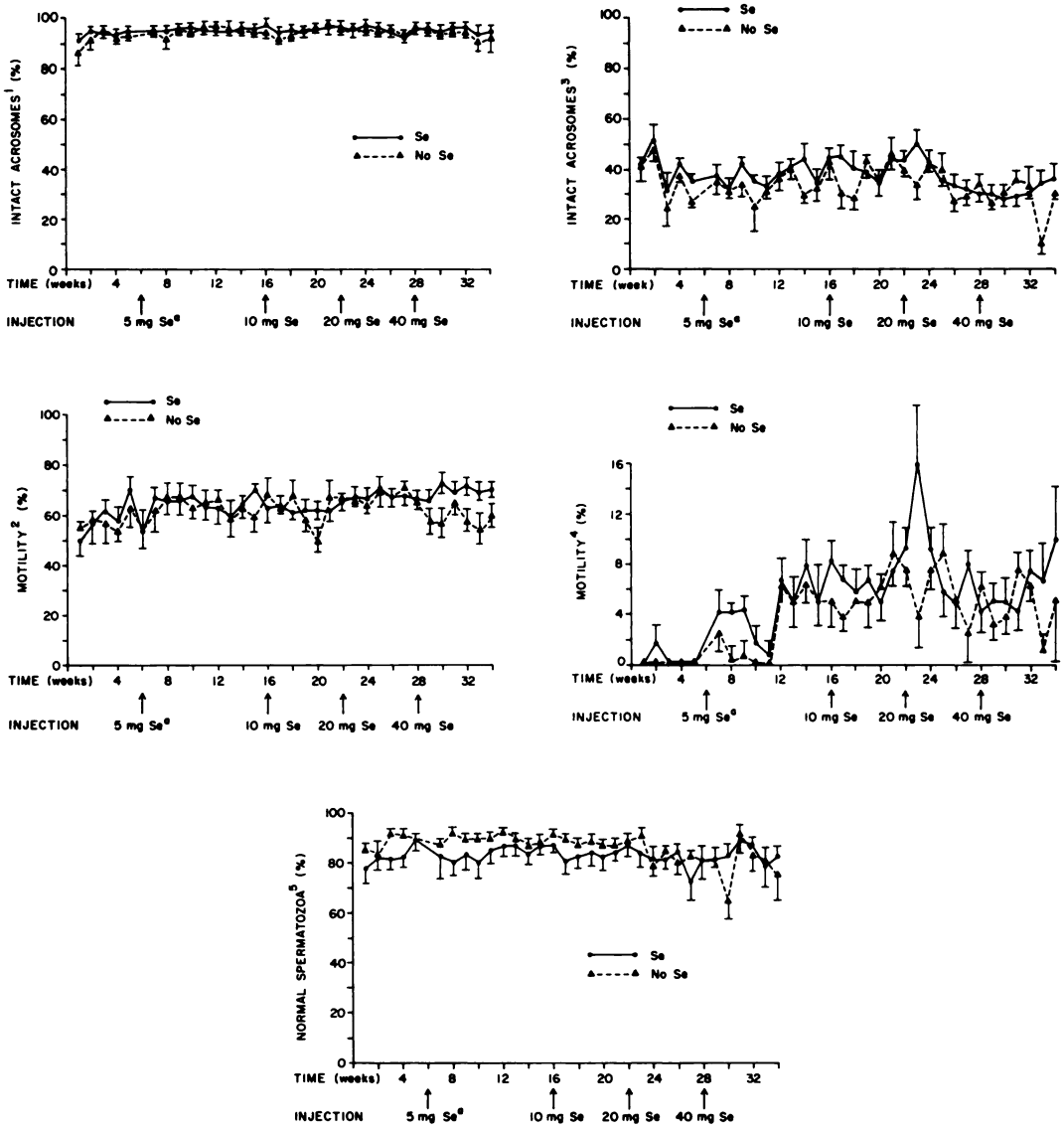

FIG. 4. Influence of Se injections on seminal quality. Each point for Se treatment represents the mean ± SEM for three bulls. Each point for no Se treatment represents the mean ± SEM for two bulls.

[a] All injections were as Na selenite per 90 kg BW.
[1] Determined postcollection.
[2] Determined postcollection.
[3] Determined post-thaw.
[4] Determined post-thaw.
[5] Determined postcollection.

to an increase in sperm numbers. The increase in semen Se was due at least in part to the increase in seminal plasma GSH-Px because the enzyme increased significantly in the Se-treated bulls. However, the increases in seminal plasma GSH-Px appeared dose-responsive (Fig. 3) and no such response was observed in semen Se (Fig. 1). Therefore, it is doubtful that seminal plasma GSH-Px accounted for all of the increase in semen Se. The following possibilities exist for the semen Se increase: 1) increased Se per spermatozoon; 2) increased

seminal plasma GSH-Px; 3) increased nonspecific association of Se with seminal plasma proteins; 4) increase in an unknown seminal selenoprotein; and 5) increased free selenium. Further research is needed to determine the cause of the high Se found in the semen of injected bulls.

Blood GSH-Px activity like blood Se, did not increase until 12 weeks after the first injection. Although Se injection significantly increased blood GSH-Px activity, the increase was not observed until after bulls received 10 mg Se/90 kg BW. In lambs, erythrocyte GSH-Px activity increased within only 1 week following dietary Se supplementation (0.51 ppm) (Oh et al., 1976). Little et al. (1979) reported erythrocyte GSH-Px activity increased significantly within 15 days following Se injection (4.5 mg sodium selenate/90 kg BW^4) in cows. In the present study, however, significant increases in blood GSH-Px activity were not observed until 12 weeks following the 5 mg injection, which was 2 weeks following the 10 mg injection. In light of these results and previous reports, it appears that the 5 mg injection was not enough to elicit a blood GSH-Px response, or an increase in enzyme activity would have been observed within the first few weeks following the 5 mg injection. Following the 10 mg injection, blood GSH-Px activity continued to increase until termination of the experiment. Oh et al. (1976) reported erythrocyte and pancreas GSH-Px activity in lambs did not peak during the duration of their experiment (2 months) although GSH-Px activity in all other tissues examined peaked when tissue Se concentration reached 0.11 ppm Se.

The changes in seminal plasma GSH-Px as a result of Se injections were especially interesting. Peaks in activity followed each injection, and each increase in Se dose produced an even higher level of GSH-Px activity. The decline in GSH-Px following each peak is without explanation, particularly the rapid decline observed between 28 and 32 weeks. It appears that the accessory sex glands are more responsive than blood to changes in Se status since 5 mg Se/90 kg BW increased seminal plasma GSH-Px but not blood GSH-Px. Synthesis of the majority of the GSH-Px in seminal plasma probably occurs in the accessory sex glands since little enzyme activity has been found in bovine epididymal samples (Brown and Senger, 1977) or mature spermatozoa (Smith et al., 1979). The accessory sex glands of the bull have been shown to incorporate selenium. Twenty-three days following Se injection, prostate and seminal vesicles contained levels of ^{75}Se surpassed only by those in the testis, epididymis, and kidney (Smith et al., 1979). Whole-body autoradiography of mice revealed the seminal vesicles contained the highest concentration of ^{75}Se of all tissues 4 days following injection (Hansson and Jacobsson, 1966).

It is important to note that bulls used in this study were diagnosed as having low blood Se (<0.05 ppm) at the outset of the experiment. Although the Se requirement for dairy bulls has not been established, Trinder et al. (1973) reported blood Se levels of less than 0.061 to 0.073 ppm in dairy cows as being deficient. Despite the apparent state of Se deficiency in these bulls, Se injections did not significantly improve semen production or spermatozoal quality as measured in this study. One of the treated bulls consistently produced a high percentage of nuclear vacuoles (10 to 50%) throughout the experiment. Thus, it appears that this morphological abnormality cannot be alleviated by Se injections. It is possible that the effects of Se deficiency on bovine male reproduction may not become evident unless the Se deficiency occurs over more than one generation as has been shown to be the case in rats (McCoy and Weswig, 1969; Halverson, 1974; Wu et al., 1973).

This study was not intended to define the recommended dose of Se for reproductively active bulls. Caution should be exercised when interpreting responses as they relate to specific dosages, since increasing doses of Se were sequentially administered and residual Se may have been present throughout the experiment. Nevertheless, it is clear that Se causes marked elevation in blood and semen Se and GSH-Px. It also appears that the bovine male reproductive system has a high affinity for Se and therefore may be a preferred organ system in Se metabolism. The possibility remains that optimum Se status was not achieved for reproductively active bulls. Further work is needed to determine the exact function of Se in the bull.

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