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Effect of Selenium Supplementation on Spermatogenic Cells of Goats

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ABSTRACT

Selenium is an essential trace mineral that is required for many physiological functions in animals and the potential relevance of selenium to the reproductive system of livestock has been considered by many researchers. The objective of this study was to examine the effect of selenium supplementation on the spermatogenic cells of goat. Eight young male crossbred (Katjang x Boer) goats, aged between 9 to 11 months, were used in this study. The control group (CON; n = 4) was fed with a diet consisting of 60% Guinea grass and 40% concentrates while the treatment group (Se-SUP; n = 4) was fed with the same diet as the goats in the control group but with supplementation of 0.6mg selenium (sodium selenite powder) per goat daily for 100 days and were slaughtered on the 101st day. There were no significant differences ($p>0.05$) in the mean number of spermatogonium, spermatocytes, spermatozoa and the total number of spermatogenic cells between the CON and Se-SUP goat respectively. However, there was a significant increase ($p<0.05$) of spermatid in Se-SUP goats. The mean percentage of spermatids was significantly increased ($p<0.05$) while spermatozoa was significantly decreased ($p<0.05$) in Se-SUP goats. In conclusion, selenium supplementation increased the percentages of spermatids and decreased the percentages of spermatozoa in the seminiferous tubules in goats.

Keywords: Goats, reproductive performance, selenium, spermatogenic cells

INTRODUCTION

Selenium is required in animals for the function of a number of selenium-dependent enzymes, also known as selenoproteins. The biochemical reactions catalysed by mammalian selenoproteins are mainly in the antioxidant defense systems, thyroid hormone metabolism and redox control of cell reactions (McKenzie, Arthur & Beckett, 2002). The overall effects of selenium on the metabolism can be associated with more specific processes that will affect the immune system (Arthur, McKenzie & Beckett, 2003).

The potential relevance of selenium to the reproductive system of livestock, laboratory animals and humans has been considered by many researchers. The health of the reproductive system of livestock is of vital importance to achieve high reproductive performance. Reproduction is one of the most important production parameters in attaining profitability in a commercial farm operation.

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Selenium is essential for the maintenance of male fertility (Brown & Arthur, 2001) and is required for testosterone biosynthesis, and for formation and normal development of spermatozoa (Behne, Weiler & Kyriakopoulos, 1996). Both the testis and epididymis require exogenously supplied selenium in order to synthesise a variety of selenoproteins (Shalini & Bansal, 2007). However, the role of these selenoproteins in spermiogenesis and post-testicular sperm maturation are not clearly defined.

Selenium deficiency has been shown to result in bilateral atrophy of the testes of rats (Behne *et al.*, 1996). Lower sperm motility, a higher percentage of abnormal sperm and lower fertilisation rate of oocytes have also been documented in boars deficient in selenium (Marin-Guzman *et al.*, 1997).

Research information on selenium supplementation for goats is lacking. No studies have been done so far to ascertain the effects of selenium supplementation on the reproductive performance of male goats. Therefore, the objective of this study was to examine the effects of selenium supplementation on the spermatogenic cells of goats. This study will also serve to determine if selenium supplementation is beneficial to the reproductive performance of male goats.

MATERIALS AND METHODS

Animals

Eight young male local crossbred (Katjang x Boer) goats, aged between 9 to 11 months, were used in this study. The control group (CON; n = 4) was fed with a diet consisting of 60% Guinea grass and 40% concentrates (palm kernel cake, rice bran and corn), while the treatment group (Se-SUP; n = 4) was fed with the same diet as the goats in the control group but with supplementation of 0.6mg selenium (sodium selenite powder) per goat daily (Liesegang *et al*., 2008). The diets were fed to the goats for 100 days and the goats were slaughtered on the 101st day.

Specimen collection and fixation

The testes were removed after slaughter and fresh testis samples were taken from the mid-region near the rete testis (Yaakub *et al*., 2009). The samples were immediately fixed in Bouin's solution for 16 hours to maintain its normal shape, and then immersed in 70% ethanol three times, one hour each time. Finally, the samples were trimmed to a thickness of 2 to 3 mm and inserted in cassettes and embedded in paraffin block.

Histological study

The paraffin block, containing the samples were removed from the cassettes and sectioned to a thickness of 5 µm using a microtome. Ten slides from each testis samples were randomly selected and stained with hematoxylin and eosin staining technique.

The areas of semineferous tubule from each slide were randomly subjected to a quantitative histological evaluation (Yaakub *et al*., 2009). Spermatogonia, spermatocyte, spermatid and spermatozoa were detected using an ocular graticule fixed to the eyepiece of normal light microscope (Olympus, UK). The numbers of spermatogenic cells in the graticule areas were counted. For each sample, the numbers of spermatogenic cells from a total of 5 random areas were counted to obtain the mean numbers of spermatogenic cells and mean percentages of spermatogenic cells. Each type of spermatogenic cells were divided by the total number of spermatogenic cells to calculate the percentage of each cell.

Statistical analysis

Statistical analysis of the numbers and percentages of spermatogenic cells of the testes samples was analysed using one-way analysis of variance (SPSS 17.0, 2008). The differences were considered significant if $p<0.05$.

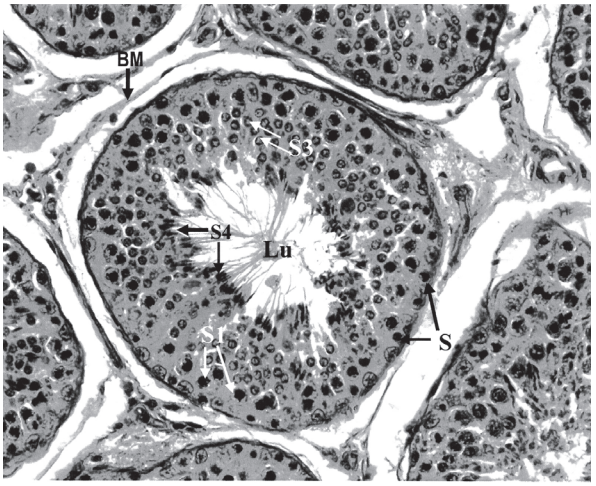

Figure 1. Histology of the seminiferous tubule of the testes from a control goat. H & E, x 400. (S: spermatogonium, S1: spermatocytes, S3: spermatids, S4: spermatozoa, BM: basement membrane, Lu: lumen)

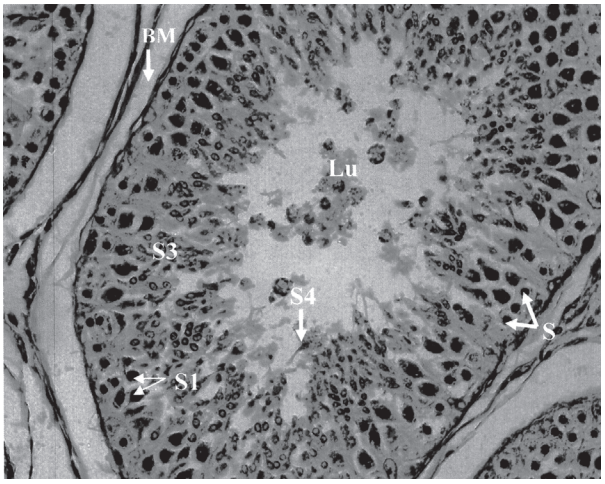

Figure 2. Histology of the seminiferous tubule of the testes from a Se-supplemented goat. H & E, x 400. (S: spermatogonium, S1: spermatocytes, S3: spermatids, S4: spermatozoa, BM: basement membrane, Lu: lumen)

RESULTS

Morphology of spermatogenic cells of goats

Figures 1 and 2 shows the microscopic morphology of the seminiferous tubule of the testes from control and Se-SUP goats. The spermatogenic cells of the seminiferous tubules of goats from both groups consist of spermatogonia, spermatocytes, spermatids and spermatozoa. Spermatogonia are located in the basal compartment of seminiferous tubules and they are small cells with densed nuclei. Spermatocytes are very large and have granular nuclei. Spermatids are small, round cells, which form clusters and occupy a position near the lumen of the

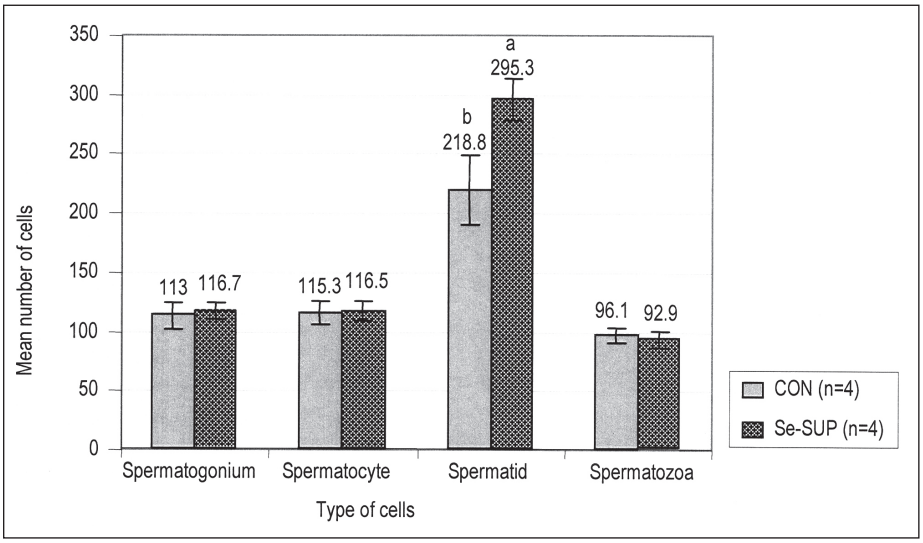

Figure 3. The mean number of spermatogenic cell counts for CON and Se-SUP goats. [a, b]Mean values with different superscripts within the same type of cells are significantly different ($p<0.05$).

seminiferous tubules. Spermatozoa have small pointed forms. Similar morphology was observed in both groups.

Mean number and percentages of spermatogenic cells

There were no significant differences ($p>0.05$) in the mean number of spermatogonium (113.0 ± 10.26; 116.7 ± 6.12), spermatocytes (115.3 ± 8.65; 116.5 ± 7.65), spermatozoa (96.1 ± 5.08; 92.9 ± 6.29) and the total number of spermatogenic cells (543.1 ± 47.39; 621.3 ± 29.99) between the CON and Se-SUP goat, respectively. However, there was a trend of increase in the mean number of spermatogonia, spermatocytes and a significant increase ($p<0.05$) of spermatids in Se-SUP (295.3 ± 16.42) compared to CON (218.8 ± 28.82) goats (Figure 3).

The mean percentage of each type of spermatogenic cells gave the same results as the mean number of spermatogenic cells, except for the spermatozoa (Table 1). The mean percentage of spermatid was significantly increased ($p<0.05$) in Se-SUP goats. However, the mean percentage of spermatozoa was significantly decreased ($p<0.05$) in Se-SUP goats. The selenium supplementation to goat increased the mean percentage of spermatid by 7% and reduced the mean spermatozoa in the seminiferous tubules by 2.9%.

DISCUSSION

The insignificant differences of the mean numbers of spermatogonium, spermatocytes and spermatozoa between the CON and Se-SUP goats imply that selenium supplementation does not affect the numbers of spermatogenic cells of goats. Therefore, selenium supplementation may not be beneficial to the reproductive performance of male goats. This finding is in agreement with the findings by Segerson & Johnson (1980), which showed that concentration of spermatozoa in the testis did not differ significantly between control and Se-supplemented bulls.

However, a trend of increased mean spermatogenic cell counts in Se-SUP goats can be observed for spermatogonium,

Table 1. Mean percentage of spermatogenic cells in control (CON) and selenium supplementation (Se-SUP) in goats

Type of spermatogenic cells	*CON (n=4)*	*Se-SUP (n=4)*
Spermatogonium	20.9 ± 1.23 [a]	18.9 ± 0.43 [a]
Spermatocyte	21.5 ± 1.23 [a]	18.8 ± 1.21 [a]
Spermatid	39.7 ± 2.09 [b]	47.4 ± 0.72 [a]
Spermatozoa	17.9 ± 0.97 [a]	15.0 ± 0.53 [b]
Total spermatogenic cells	100.0	100.0

[a b] Mean values with different superscripts between columns are significantly different ($p<0.05$)

spermatocytes and spermatids. The increase in the numbers of spermatids is especially more prominent and may imply that selenium is required in the pathway where secondary spermatocytes undergo second meiotic division to form spermatids.

The analysis on the percentages of spermatogenic cells showed significant increases in the percentages of spermatids for Se-SUP goats. This, together with the increased mean cell counts of spermatids in Se-SUP goats, may imply that selenium is required for the formation of spermatids. A few reasons may account for the significant decreases in the percentages of spermatozoa in Se-SUP goats where selenium supplementation may be detrimental to the formation of spermatozoa or selenium supplementation may prolong the lifespan of spermatids and delay spermiogenesis. Selenium is required in the biosynthesis of testosterone and selenium supplementation may increase the levels of testosterone, which increases the rate of maturation of the spermatozoa (Jana *et al*., 2008; Behne *et al.*, 1996). This leads to increased rate of spermiation (i.e. process where mature spermatozoa are released from the protective Sertoli cells into the lumen of the seminiferous tubules) and rapid transport of the non-motile spermatozoa to the epididymis, which may account for the decreased percentages of spermatozoa in the seminiferous tubules. Further studies should be conducted to investigate the role of selenium in spermatogenesis and spermiogenesis.

Behne *et al*. (1996) showed that selenium deficiency resulted in the lack of stem cells in the seminiferous tubules of rats. The seminiferous tubules had reduced diameters and no differentiated spermatozoa were detected. In this study, the diameters of the seminiferous tubules of the goats were not measured. The numbers of spermatogenic cells were not affected by selenium treatment in this study. It appears that both the CON and Se-SUP goats had sufficient dietary selenium to prevent any alteration in the morphology of the spermatogenic cells.

These inconsistent results in animals may reflect species differences in the utilisation of selenium (Jana *et al*., 2008; Behne *et al.*, 1996). Selenium may be required for the spermatogenic function of the testes of rats but not for the spermatogenic function of the testes of goats.

The differences in the forms of selenium fed to the experimental animals may account for the differences in the results between this study and in previous studies on the reproductive performance of animals (Kaur & Bansal, 2005, El-Sisy *et al.*, 2008). This may be due to different forms of selenium supplements that have different levels of bioactivity. Sodium selenite powder was used in this study. Sodium selenite and sodium selenate are chemically inorganic supplemental sources which come as trace mineral salts with selenium. Both selenite

and selenate are considered to have 100% bioactivity in ruminants and the usage of trace mineral salts with selenium is the least expensive way to increase selenium intake in animals (Schivera & Sideman, 2007). However, organic forms of selenium supplements, such as selenomethionine and selenised yeast, have higher levels of bioactivity when compared to inorganic supplements (Schivera & Sideman, 2007).

In addition, nutrition generally affects the endocrine rather than the spermatogenic function of the testis (Jainudeen &Hafez, 2000). Segerson & Johnson (1980) showed that the number of spermatozoa did not differ significantly between control and selenium supplemented bulls despite the increase in selenium concentration in the testis of selenium supplemented bulls over that in the testis of control bulls. This suggests that the increase in selenium concentration in the testis was not associated with the spermatogenic elements in the testis.

The current study showed no significant differences in the total number of spermatogenic cells between the CON and Se-SUP goats. The lack of selenium supplementation effects in this study may be due to the fact that selenium supplementation to deficient diets will often elicit a positive response, whereas additional supplementation to selenium-adequate diets would not be expected to produce additional clinical benefits (Munoz *et al.*, 2009; Seboussi *et al.*, 2009). The diet fed to the goats in this study may already contain adequate selenium and thus, additional supplementation did not show significant results. However, our study shows that 0.6mg selenium supplementation as selenate powder, increased the percentages of spermatids but decreased the percentages of spermatozoa in the seminiferous tubules in goats.

It cannot be denied that selenium supplementation in selenium-deficient diets is important as selenium plays important roles in many other physiological processes. Therefore, selenium supplementation is necessary for the health and normal physiological functions of animals. Although there have not been extensive studies on the selenium contents in the soil and in the diets of the animals in Malaysia, selenium deficiency in the soils and forages is very likely because of the high levels of rainfall in Malaysia. This could lead to leaching of selenium from the soil and the dilution of selenium taken up by more prolific plant growth when rainfall is high (Rudolph, Andreller & Kennedy, 2008). Further studies should be conducted to measure the selenium in the soil and diets, prior to supplementation.

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