Selenium supplementation prevents DNA damage in ram spermatozoa

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ABSTRACT: In the present study, we aimed to evaluate the effects of different concentrations of selenium (Se) ovine nutritional supplementation on spermatozoa DNA integrity. Thirty male ovines (age: 10 months) were used. They were fed with hay and ram food in an intensive system, which was divided into stalls (5 m long and 3 m wide) with feeding troughs, and had ad libitum access to food and water. Ovines in group 1 (G1, the negative control) received mineral salt supplementation without Se; ovines in G2 received the same mineral salt mixed with 5 mg Se (as sodium selenite)/kg mineral supplement; ovines in G3 received 10 mg Se/kg mineral supplement; ovines in G4 received 15 mg Se/kg mineral supplement; and ovines in G5 received 20 mg Se/kg mineral supplement. Ovines in all groups remained untreated for 14 days, followed by a treatment period of 56 days. Semen samples were obtained by electroejaculation. The DNA damage in semen samples was evaluated using the comet assay. The mean differences were compared using Tukey’s test at a significance level of 5%. The control group (G1) showed a high percentage of DNA damage compared to the Se-treated groups (G2–G5). Therefore, Se supplementation could decrease the basal level of DNA damage in sperm cells, suggesting that Se might exert protective effects on sperm DNA.

Key words: chemoprevention, comet assay, semen, ovine.

INTRODUCTION

Sheep industry represents an important productive sector of wool for garments, meat, dairy, and leather for human consumption (SCHWAB, 2011). In an ovine herd, the ram is mainly responsible for genetically improving the stock, owing to its capacity to mount more than 35 females under natural conditions during a breeding season (MANDIKI et al., 1998). Therefore, selection of ram breeders...
with high fertility is important for ovine production (MATOS et al., 1992). In this context, the assessment of sperm DNA integrity is quite relevant, wherein fertile sperms must have a stable DNA capable of decondensation at the appropriate time of fertilization, while maintaining the genetic codification without mutations (AGARWAL & ALLAMANENI, 2005).

Among the essential trace minerals, selenium (Se) represents a key element for spermatogenesis and male fertility, where it improves reproductive health (BOITANI & PUGLISI, 2009, HAWKES & TUREK, 2001). The main sources of Se routinely used for animal supplementation are sodium selenite and selenate as inorganic Se, and Se-enriched yeast (Se-yeast) as organic Se (PETRERA et al., 2009). However, studies on the effects of this micronutrient and related minerals on ovine reproduction are still scarce. Therefore, the investigation of genetic changes in ram sperm is important for ovine herd improvement. Herein, we used the comet assay (single-cell gel electrophoresis assay) to evaluate the effects of Se-enriched diets on ram sperm DNA integrity. Comet assay is a short-duration and low-cost technique that can detect single- and double-strand breaks and alkali-labile sites in DNA (KOPPEN et al., 2017).

In reference to the above, the present study aimed to evaluate the effects of different concentrations of selenium (Se) ovine nutritional supplementation on spermatozoa DNA integrity.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee (Protocol 180/2014) at the São Paulo State University – UNESP, Botucatu, São Paulo State, Brazil. The experimental conditions, endoparasite and ectoparasite vaccine control, and geographic location of the present study were the same as described by PIAGENTINI et al. (2017).

The experimental design of this study was developed through 5 × 5 Latin square design, with five treatments and five repetitions for the experimental period. Thirty ten-month-old, sexually mature rams (*Ovis aries*) (HULET & SHELTON, 2004) were distributed randomly into five groups (n = 6) and fed hay and ram food (Allnova Fanton Animal Nutrition Industry, Brazil) in an intensive system, which was divided into masonry stalls (5 m long and 3 m wide) with feeding troughs and provided *ad libitum* access to food and water. Ovines in group 1 (G1; the negative control) received mineral salt supplementation without Se; ovines in G2 received the same mineral salt mixed with 5 mg Se (sodium selenite)/kg mineral supplement; ovines in G3 received 10 mg Se/kg mineral supplement; ovines in G4 received 15 mg Se/kg mineral supplement; and ovines in G5 received 20 mg Se/kg mineral supplement. Ovines in all groups remained untreated for 14 days, followed by a treatment period of 56 days. Selenium concentrations used in this experiment were based on the studies by LANGLANDS et al. (1990) and UNDERWOOD & SUTTLE (1999).

Both sodium selenite and the commercial mineral diet were purchased from Ovisal® (Allnova Fanton Animal Nutrition Industry, Brazil). The commercial mineral diet contained the following concentrations of the elements per kg: 89 g calcium, 45 g phosphorus, 1,98 g sulfur, 2.5 g magnesium, 2,160 mg zinc, 300 mg manganese, 120 mg cobalt, 8 mg iodine, 97.5 g sodium, and 450 mg fluoride (maximum), with a P205 solubility in citric acid of 95 mg (minimum). This mineral supplement was supplied at 30 g/animal/day, as recommended by the manufacturer.

Blood samples were collected after each treatment period. The serum Se concentration in ovines was determined by an atomic absorption technique using a graphite furnace. The semen was collected by electroejaculation after each treatment period. Seminal characteristics of the ejaculate (volume, mass movement, total motility, vigor, and sperm concentration) were analyzed according to the recommendations of CBRA (1998). Sperm morphology evaluation was carried out using a stained semen smear, following the modified Karras method (PAPA et al., 1988) and evaluated under an optical microscope according to the classification proposed by BLOM (1973).

All ovines in the five groups consumed all mixtures during the five periods of 56 days each. The amount of Se from the daily consumption of mixtures increased as each animal consumed the corresponding amounts daily. On day 0 (D0), the ovines were weighed, and samples of semen were collected for preliminary analysis before treatment. The total period of supplementation was 280 days, i.e., 5 cycles of ram spermatogenesis (56 days/each cycle), with 14 days intervals between the treatment periods, as previously described by HAFEZ & HAFEZ (2004).

Sperm DNA fragmentation was assessed by the neutral comet assay, as described by LINFOR & MEYERS (2002) and modified for ram sperms by MARTINS et al. (2013). For the analysis, the slides (in duplicate) were stained with SYBR® Gold aqueous

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selenium solution (1:10,000; Invitrogen, USA) and 100 randomly selected nucleoids were scored using a fluorescence microscope connected to an image analysis system (Comet Assay IV; Perceptive Instruments, UK). The extent of DNA damage was expressed as tail intensity (% of tail-migrated DNA). Slides treated with 10% H$_2$O$_2$ served as a positive control.

A one-way analysis of variance (ANOVA) with Tukey’s post hoc test was used to analyze the differences in tail intensity among the experimental groups, with $P<0.05$ considered statistically significant. All data were analyzed using the GLM procedure in SAS.

RESULTS AND DISCUSSION

The sexual maturity of a ram is determined by sperm production, semen quality, and libido (KINBERLING & PARSONS, 2007). According to HULET & SHELTON (2004), ram maturity is reached at six months of age. Thus, we selected animals at ten months of age, to ensure that they were sexually mature and to standardize the age of the animals in the groups. This age of the animals also corroborates with CHACÓN et al. (2019), who reported when analyzing spermatozoa concentration and progressive motility during ram lamb development that the semen quality variables improved from six months until ten months of age, when value stabilization occurred and remained uniform until 12 months old.

The mean serum selenium concentration was significantly lower in G1 (15.46 ± 0.91 μg/dL) than in the other treatment groups (G2 = 20.23 ± 0.99 μg/dL, G3 = 20.79 ± 0.94 μg/dL, G4 = 19.94 ± 0.92 μg/dL and G5 = 21.33 ± 0.94 μg/dL) due to the mineral supplementation. Low-Se diets can favor DNA lesions in the spermatozoan genome, with potential implications for the offspring health (GRAUPNER et al., 2015). Selenium is a micronutrient that is necessary for testosterone biosynthesis and the normal formation and development of spermatozoa. Thus, Se deficiency can affect the testicular morphology and functions (BEHNE et al., 1996).

After each semen collection, the following spermatologic parameters were analyzed: volume, mass movement, motility, vigor, spermatologic concentration, and sperm morphology. All the rams exhibited values within the established standards for the species (CBRA, 1998). Results of the semen analysis were described in table 1. As for sperm evaluation, there was no statistical difference among the treatment groups in terms of the volume, mass movement, total motility, vigor, and sperm concentration ($P>0.05$). Sperm morphology differed among the treatment groups; G1 (0 mg of selenium) had the highest percentage of major defects ($P<0.05$). The Se-supplemented diets (5, 10, 15, and 20 mg/kg mineral mix/animal/day) reduced the sperm morphological abnormalities that are classified as major defects, including a strongly folded tail, as well as acrosome and head defects, thereby confirming the results of BECKETT & ARTHUR (2005). However, the percentage of minor defects did not show a statistically significant difference among the treatments ($P>0.05$). Conversely, no changes in sperm volume, mass movement, total motility, vigor, and concentration were detected.

Sperm morphology usually correlates with the genetic integrity (SAID et al., 2005, CARREIRA

| Table 1 - Mean values ± the standard error of sperm parameters including the seminal volume, mass movement, total motility, vigor, sperm concentration, and sperm defects (major and minor) of rams treated with Se (sodium selenite as inorganic Se) at 5, 10, 15, and 20 mg Se/kg mineral mix/animal/day. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sperm parameters               | G1              | G2              | G3              | G4              | G5              |
| Volume (mL)                     | 1.13±0.10$^a$  | 1.33±0.09$^a$  | 1.27±0.10$^a$  | 1.26±0.09$^a$  | 1.21±0.10$^a$  |
| Mass movement                   | 4.67±0.10$^a$  | 4.48±0.11$^a$  | 4.43±0.10$^a$  | 4.49±0.11$^a$  | 4.80±0.10$^a$  |
| Total motility (%)              | 87.06±1.17$^a$ | 85.23±1.20$^a$ | 83.87±1.16$^a$ | 84.84±1.17$^a$ | 88.14±1.16$^a$ |
| Vigor                           | 4.60±0.09$^a$  | 4.52±0.10$^a$  | 4.49±0.09$^a$  | 4.49±0.10$^a$  | 4.81±0.09$^a$  |
| Concentration (×10$^6$)         | 1.31±0.17$^a$  | 1.88±0.16$^a$  | 1.50±0.16$^a$  | 1.29±0.15$^a$  | 1.41±0.16$^a$  |
| Major defect                    | 11.11±1.11$^a$ | 6.93±1.17$^a$  | 7.76±1.11$^a$  | 6.39±1.12$^a$  | 5.05±1.13$^a$  |
| Minor defect                    | 5.91±0.65$^a$  | 6.89±0.69$^a$  | 6.48±0.65$^a$  | 6.32±0.64$^a$  | 6.07±0.67$^a$  |

Tukey test ($P<0.05$).
et al., 2015). Sperm DNA fragmentation has emerged as a potential causative factor of reproductive failure, and its assessment has been used as a novel laboratory tool to investigate male infertility (EVENSON, 2016, EVGENI et al., 2014). Our data showed a decrease in the spermatozoa DNA damage for Se-supplemented diet-fed animals at all tested concentrations (5, 10, 15, and 20 mg/kg mineral mix/animal/day) (Figure 1). Therefore, the concentration of 5 mg of Se may be the minimum amount necessary to protect sperms during their formation. However, no animal showed any clinical signs of intoxication.

The chemopreventive effects of Se might be related to selenoproteins. Selenium is a trace and crucial element that was incorporated into selenoproteins, which have catalytic activity and act as an antioxidant (glutathione peroxidase, GPx). Recently, GRAUPNER et al. (2015) showed that selenoproteins might reduce the formation of DNA lesions. Several studies have shown that reactive oxygen species (ROS) overproduction is the main cause of apoptosis and subsequent DNA fragmentation. As an antioxidant, Se neutralizes the excessive ROS, resulting in less damage to sperm DNA (WANG et al., 2003, DOROSTKAR et al., 2012). However, despite the substantial progress made towards understanding the protective role of Se, its molecular mechanisms of action are still unknown.

Figure 1 - DNA damage (tail intensity) in the sperm cells of rams treated with Se (sodium selenite as inorganic Se) at 5, 10, 15, and 20 mg/kg mineral mix/animal/day (A). Different letters indicate significant differences among groups (ANOVA with Tukey’s post hoc test; \( P < 0.05 \)). Photomicrographs showing nucleoids of sperm cells from (B) positive (10% \( \text{H}_2\text{O}_2 \)) and (C) negative (diet without Se) controls at 400X magnification.
This paper is an initial study about the beneficial role of Se supplementation to ovine, and more studies should be carried out to evaluate the effects of this supplementation on fertility.

CONCLUSION

In conclusion, Se-supplemented diets were associated with reduced DNA damage in ram sperm cells. However, further studies using other genetic endpoints and test systems are necessary for improving our understanding of the mechanisms of Se action as a chemopreventive agent for safely maintaining ram sperm DNA integrity.

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AUTHORS’ CONTRIBUTIONS

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