



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 experimental design was implemented using a 5 × 5 Latin Square, i.e., five treatments and five experimental periods. 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.

Suplementação com selênio previne danos ao DNA espermático de ovinos

RESUMO: *O presente estudo teve por objetivo avaliar os efeitos da suplementação mineral com diferentes concentrações de selênio (Se) sobre a integridade de DNA espermático de ovinos. Utilizaram-se 30 machos, com 10 meses de idade. Eles foram mantidos em sistema intensivo, sendo alimentados com feno e ração própria para ovinos, divididos em baias (5 m x 3 m), com cochos e água ad libitum. Os ovinos do grupo 1 (G1=controle negativo) receberam suplementação de sal mineral sem a adição de Se, os animais do G2 receberam a mesma mistura mineral, porém com 5 mg de Se (selenito de sódio)/kg mistura mineral, os ovinos do G3 receberam 10 mg Se/kg mistura, os animais do G4 receberam 15 mg Se/kg mistura, os do G5 receberam 20 mg Se/kg mistura. Os ovinos de todos os grupos passaram por um período de adaptação de 14 dias, seguido por um período de tratamento de 56 dias. O sêmen foi colhido por meio de eletroejaculação. A integridade do DNA espermático foi avaliada por meio do teste de cometa. O modelo experimental utilizado foi Quadrado Latino 5 x 5, com cinco grupos e cinco períodos experimentais. A diferença entre as médias foi analisada pelo teste de Tukey, com 5% de nível de significância. O grupo controle (G1) apresentou elevada porcentagem de danos quando comparada aos demais grupos de tratamentos (G2 a G5). Portanto, a suplementação de Se diminui o nível de danos ao DNA espermáticos, sugerindo que o Se pode exercer efeitos protetores sobre o DNA dos espermatozoides de ovinos.*

Palavras-chave: quimioprevenção, teste do cometa, sêmen, ovino.

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

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% H₂O₂ 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 ($15.46 \pm 0.91 \mu\text{g/dL}^b$) than in the other treatment groups (G2 = $20.23 \pm 0.99 \mu\text{g/dL}^a$, G3 = $20.79 \pm 0.94 \mu\text{g/dL}^a$, G4 = 19.94 ± 0.92

$\mu\text{g/dL}^a$ and G5 = $21.33 \pm 0.94 \mu\text{g/dL}^a$) 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 spermiatic parameters were analyzed: volume, mass movement, motility, vigor, spermiatic 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 \pm 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.

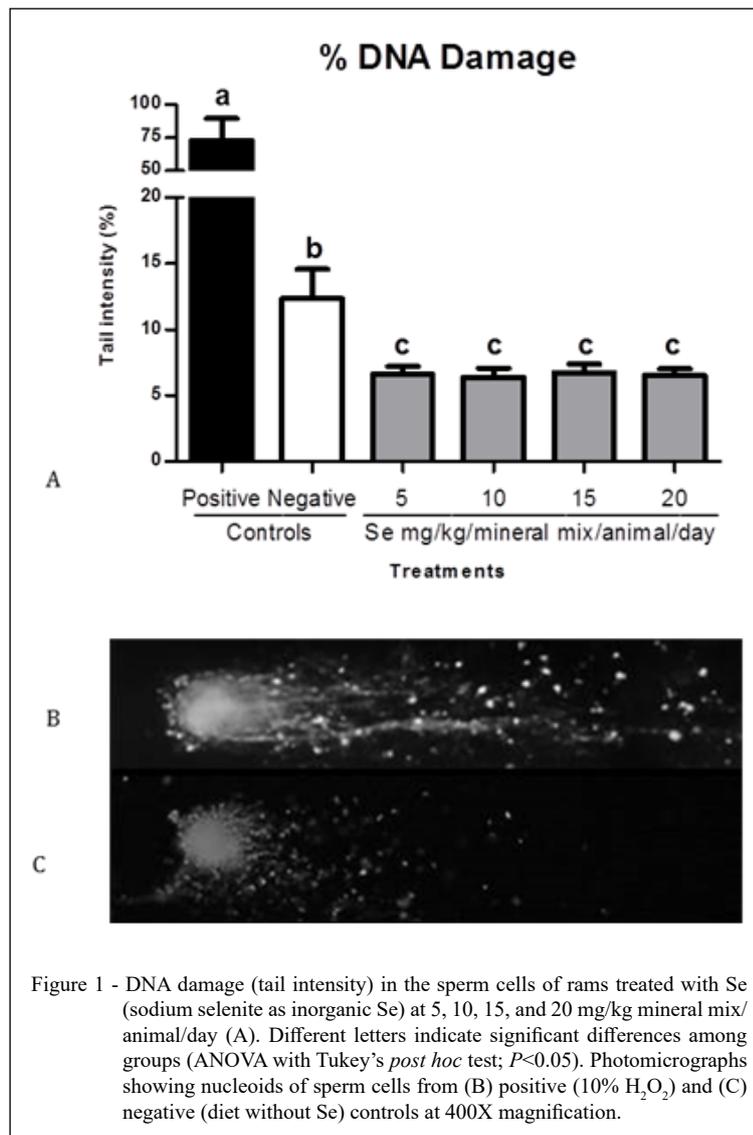
Sperm parameters	Group				
	G1	G2	G3	G4	G5
Volume (mL)	1.13 \pm 0.10 ^a	1.33 \pm 0.09 ^a	1.27 \pm 0.10 ^a	1.26 \pm 0.09 ^a	1.21 \pm 0.10 ^a
Mass movement	4.67 \pm 0.10 ^a	4.48 \pm 0.11 ^a	4.43 \pm 0.10 ^a	4.49 \pm 0.11 ^a	4.80 \pm 0.10 ^a
Total motility (%)	87.06 \pm 1.17 ^a	85.23 \pm 1.20 ^a	83.87 \pm 1.16 ^a	84.84 \pm 1.17 ^a	88.14 \pm 1.16 ^a
Vigor	4.60 \pm 0.09 ^a	4.52 \pm 0.10 ^a	4.49 \pm 0.09 ^a	4.49 \pm 0.10 ^a	4.81 \pm 0.09 ^a
Concentration (x10 ⁹)	1.31 \pm 0.17 ^a	1.88 \pm 0.16 ^a	1.50 \pm 0.16 ^a	1.29 \pm 0.15 ^a	1.41 \pm 0.16 ^a
Major defect	11.11 \pm 1.11 ^a	6.93 \pm 1.17 ^b	7.76 \pm 1.11 ^b	6.39 \pm 1.12 ^b	5.05 \pm 1.13 ^b
Minor defect	5.91 \pm 0.65 ^a	6.89 \pm 0.69 ^a	6.48 \pm 0.65 ^a	6.32 \pm 0.64 ^a	6.07 \pm 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.



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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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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