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INFLUENCE OF SELENIUM ADDITIVE ON RAM SEMEN FREEZING QUALITY

Beata Seremak, Jan Udala, Bogdan Lasota
Department of Animal Reproduction, Agricultural University, Szczecin, Poland

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ABSTRACT

The tests were conducted on 16 eiaculates sampled from 4 rams. After sampling and assessment the semen was divided into 3 parts: I – test, II – with the addition of 1 µg Se/ml, III - with the addition of 10 µg Se/ml. The semen was made subject to a 4-hour equilibration at the temperature of 4°C, and then frozen. As a result of the conducted tests, a positive influence was proved of the applied selenium additive to semen in the quantity of 1 µg /ml on its freezing ability and survival at the temperature of 38°C.

Key words: rams, semen, selenium, freezing

INTRODUCTION

Selenium is an element fulfilling a significant function in reproduction processes, both in females and males of farm animals (8, 9, 17, 23, 27, 28). Disturbances in animal reproduction are connected with insufficient selenium supply in the organism. In females they are manifested (among others) by reduction of oestrus strength, decreased fertilizability index (7), ovary function disturbances and uterus involution course, and a more frequent placenta retention (11, 12, 22). In males bred on a low selenium diet, male hypogonadism was found as well as reduced production and deteriorated semen quality (13, 16, 24, 26). The aim of this article is to observe how the additive of selenium to semen prior to freezing influences its quality during subsequent processing phases and after defreezing.

MATERIALS AND METHODS

The tests were performed on 16 eiaculates sampled from 4 rams of Polish long-wool sheep at the age of 2 – 3 onto an artificial vagina.

After sampling and eiaculate semen assessment the samples were divided into 3 parts; then the citrate-vitelline-fructose diluent was added to each of them, prepared after Bielanski and co-authors (3):

I – with no additive of selenium (test sample)
II – with the additive of 1 μg Se/ml
III – with the additive of 10 μg Se/ml
Selenium was added as a sodium selenite.

The diluent included: a 2.9% solution of two-hydrate sodium citrate – 76 ml, yolk of hen egg – 20 ml, fructose – 1.25 g, glycerol – 4 ml.

The final glycerol concentration in the diluent amounted to 4%, and the spermatozoon concentration 0.9 x $10^9/cm^3$.

After the semen dilutiing, it was made subject to a 4-hour equilibration at the temperature of 4^oC.

Then, the semen was poured to aluminum horns, having the volume of 2 cm^3, which were frozen on a stand immersed in liquid nitrogen according to the methodology described by Ali and Tischner (1). The defreezing of semen was carried out by shifting the horns from liquid nitrogen to the water bath, having the temperature of 38 – 40^oC per 30 seconds.

After the semen defreezing, as well as earlier, during its particular processing phases, the spermatozoon motility was tested, and smears were conducted on a slide, and then contrasted with a 10% nigrosine solution in order to evaluate the spermatozoon acrosome condition. This assessment was performed according to Saake and White (18). The test of spermatozoon survival was carried out at the temperature of 38^oC according to the methodology supplied by Bielanski (2).

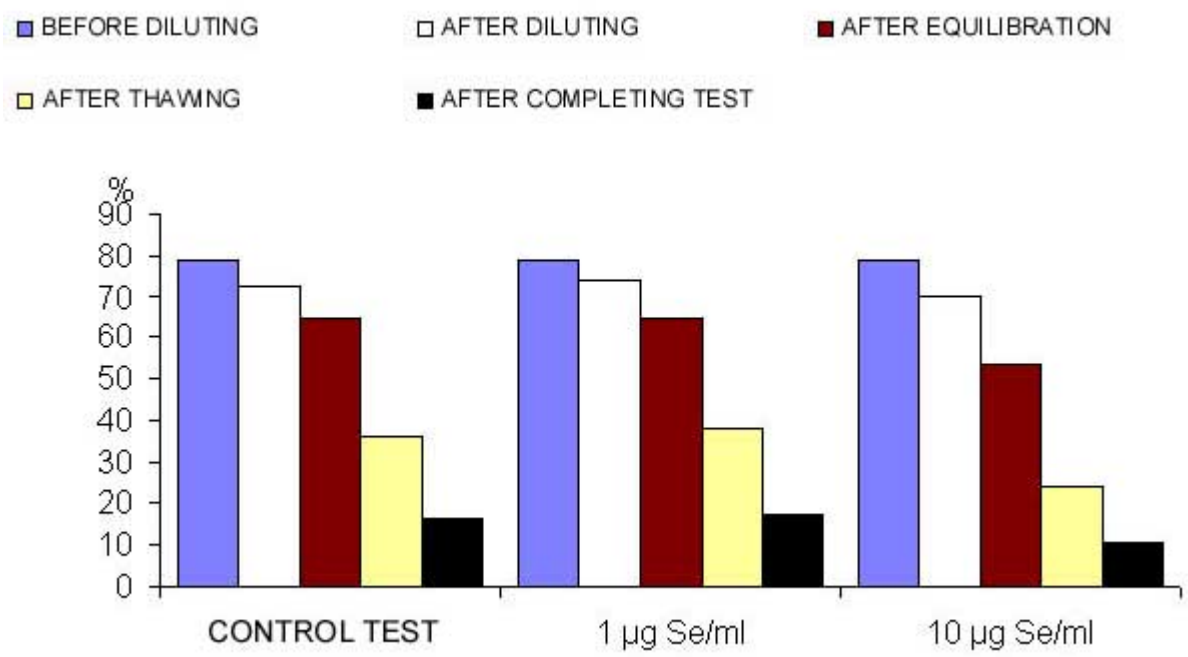
The test of spermatozoon movement was conducted immediately after the semen defreezing, and then every one hour up to the moment of observing oscillating movements only (lack of spermatozoon demonstrating the progressing movement) or the lack of movement of all spermatozoon. The achieved results were made subject to statistic analysis.

RESULTS AND DISCUSSION

The semen assigned for freezing was characterized by good quality. The mean values of assessed indexes did not diverge from the accepted standards for ram semen (2, 25). The average volume of 16 sampled eiaculates amounted to 1.1 ± 0.2 cm^3, there was on average 3.65 ± 0.59 billion spermatozoon in 1 cm^3 of semen, out of which 82.0 ± 5.7% demonstrated the progressing movement. The main morphological changes oscillated at the level of 2.6 ± 0.8%, and the subordinate ones 7.4 ± 2.6%. The spermatozoon with the undamaged acrosome constituted 82.3 ± 11.5% of all sperm cells.

It was proved that directly after the dilution of semen in a sample with no additive of selenium (group I) and with the additive of 1 µg Se/ml (group II) the percentage of spermatozoon demonstrating the progressing movement (Figure 1) was nearly identical and amounted to 74.6 ± 5.3 and 74.5 ± 3.7 respectively. As for group III, containing 10 µg Se/ml in this phase of semen processing, a several-percent spermatozoon increase showing the progressing movement was observed in comparison with the first two groups.

Figure 1. Percentage of spermatozoon demonstrating progresssing movement in particular phases of semen processing after adding different selenium doses.

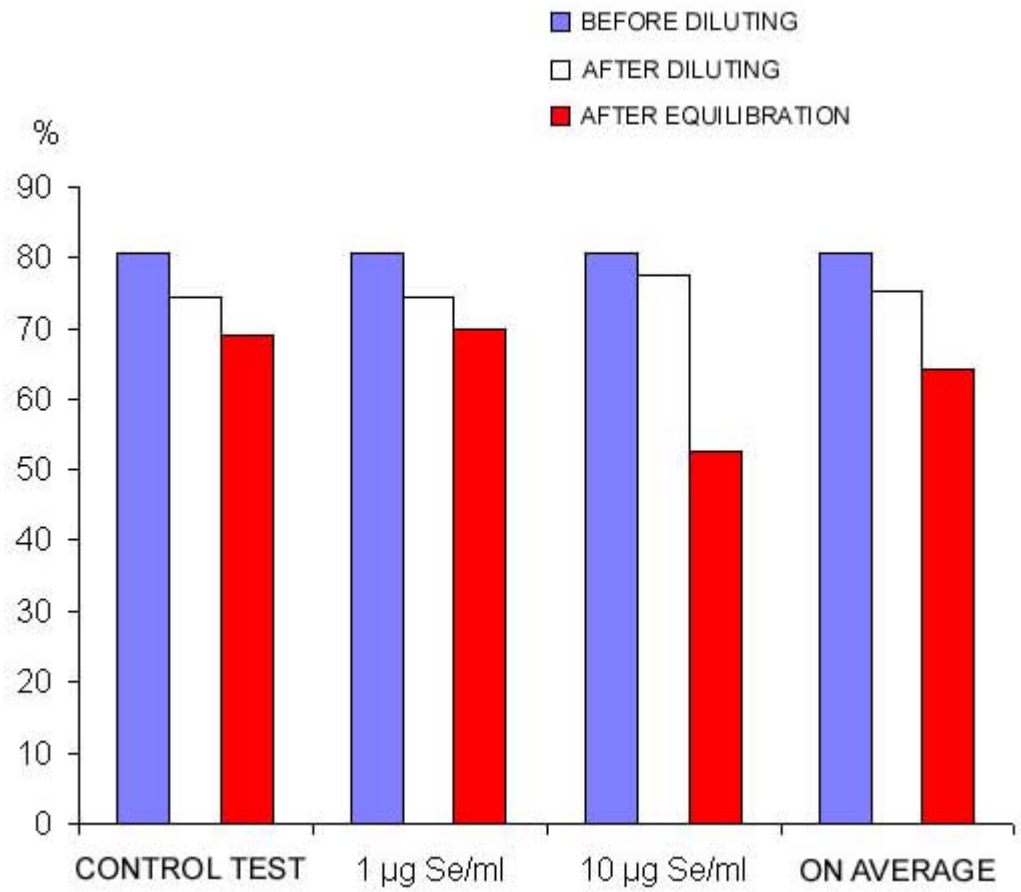


The distribution of the assessed index acquired an entirely different form in the tested samples after a 4-hour equilibration at the temperature of 4°C. In group I and II, 69.2 ± 4.3% and 70.0 ± 3.1% of spermatozoon on average demonstrated the progressing movement, while in group III the index amounted to 52.5 ± 17.1%. The estimated differences among the groups described above proved to be statistically significant. Thus, the additive of 10 µg Se/ml to the semen had a negative influence on the spermatozoon motility as early as in the sample preparation phase for freezing. This influence was most probably connected with the destructive impact of high microelement concentration on the spermatozoon insert, regarded as an energetic area of male gamete (14), and with the impairment of processes occurring in mitochondria. Mitochondria are surrounded by a keratinous capsule particularly rich in selenoprotein (5, 6, 15, 19); for this reason this element plays the main role in maintaining a

proper composition of this structure (6, 24, 26). Additional tests demonstrated that the application of a 10 µg Se/ml dose caused approx. 100-fold selenium concentration increase in the semen plasma, in comparison with values occurring normally in rams. As Saaranen and co-authors state (19), the selenium concentration in the semen plasma of the discussed animal species oscillates on average at the level of 102 ± 19.1 µg/l. The obtained results allow presuming that the commonly known toxic action of selenium applied excessively to animals 'in vivo', occurs also in reference to research on sperm cells 'in vitro'.

The acrosome morphological tests conducted in particular phases of semen processing demonstrated that the high microelement concentration had also a negative effect on the condition of this structure in semen, playing an important role in the fertilization process of the egg cell (21). As the provided data reveal (Figure 2), the degree of acrosome damage in samples where 10 µg Se/ml was added was considerably higher than in samples with a 10-fold lower element addition and in test samples. This effect was particularly noticeable after semen defreezing and after the spermatozoon survival test at the temperature of 38°C.

Figure 2. Percentage of spermatozoon with undamaged acrosome in groups of different selenium additive.



The test results, both 'in vivo' and 'in vitro', concerning the influence of selenium on the spermatozoon morphological condition, are not univocal. The research conducted by Wu and co-authors (26), Wallace and co-authors (24) on male rats and mice deficient in selenium supply demonstrated the increase of morphological changes in their semen.

Tests carried out on rodents are not always confirmed by research performed on males of other mammals. Heimann and co-authors (10) inform (among others) about the lack of univocal dependencies between the concentration of the discussed microelement in the reproductive system organs, blood and semen, and the value of morphological changes in bull spermatozoon; Buchanan-Smith and co-authors obtained similar results for a ram (4).

These tests, on the other hand, correspond with the results gained by Siegel and co-authors (20) on bull semen. The authors mentioned above proved that the additive of selenium of up to 1 μg/ml had a positive impact on the motility of fresh and frozen semen. In case of doses exceeding 2 μg/ml the authors noted a drop in spermatozoon motility, and at doses of over 5 μg/ml the negative influence of this microelement proved statistically significant. The additive of selenium in the quantity of 0.65 μg/ml proved most optimal.

Similar results of tests conducted 'in vivo' on bulls ware obtained by Udala and co-authors (23). The application of selenium with vitamin E and selenium on its own in the form of intramuscular injection significantly influenced the percentage increase of spermatozoon demonstrating the progressing movement after the semen defreezing, and eventually it caused gaining a larger number of semen portion from an eiaculate. What results from these tests is that the effect of selenium administration is particularly noticeable in animals characterized by low contents, below optimal values, of this element in their blood.

The test sampling of selenium concentration carried out additionally in applied diluents demonstrated that the concentration varied from 55 to 62 ng/ml, and in the semen plasma assigned for tests it amounted to: from 40 to 115.5 ng/ml. In case of ram semen, opposite to blood, there are no norms allowing for univocal specification what selenium level is regarded as deficient. The research of Saaranen and co-authors (19) shows that the concentration of the discussed microelement in the semen plasma is approx. 100 ng/ml. Therefore, in some semen samples used for the test, the (selenium) level was lower than the mentioned value, but it is difficult to state if it was insufficient.

The research on the semen survival indicates that the spermatozoon life span in the environment containing 1 μg Se/ml was the longest and amounted to 7.5 hours. In the test sample this span was shorter by 1 hour, and in the sample containing 10 μg Se/ml – by 3 hours on average. This parameter had the most favorable form in the semen containing selenium in the quantity of 1 μg/ml. The obtained results correspond, to a large extent, with the research of Siegel and co-authors (20) presented earlier, carried out on bull semen.

The presented tests suggest generally positive selenium influence on the quality of ram frozen semen, and they also account for the complexity of the issue. The research conducted by the authors cited above shows that the effect of this element can be various, depending on numerous factors, but their analysis was not the aim of this research. The animal species, as well as the organism supply with this element, or simply the form and way of using it play an unquestionable role here. It would be interesting to respect the relations between the discussed microelement and its antagonists. There are no research papers to be found in the available literature referring to semen.

CONCLUSIONS

The conducted research account for the purposefulness of adding 1 μg/ml to semen before its freezing. Ten-fold higher doses, though, have a negative influence on the semen freezing ability.

REFERENCES

1. Ali S.B.A., Tishner M.: Mrozenie nasienia trykow w tubach aluminiowych i proby glebokiej doszyjkowej inseminacji owiec. Med. Wet. 5, 294-297, 1988.
2. Bielanski W.: Rozrod zwierzat. PWRiL Warszawa 1977.
3. Bielanski W., Boryczko Z., Kosiniak K., Okolski A., Tischner M.: Przewodnik do zajec praktycznych z zakresu biologii rozrodu i sztucznego unasieniania zwierzat gospodarskich. AR Szczecin 1980.
4. Buchanan-Smith J.G., Nelson E.C., Osburn B.I., Wells M.E., Tillman A.D.: Effects of vitamin E and selenium deficiencies in sheep fed a purifield diet during growth and reproduction. J. Anim Sci. 29, 808, 1969.
5. Calvin H.I.: Selective incorporation of selenium 75 into a polypeptide of the rat sperm tail. J. Exp. Zool. 204, 445-452, 1978.
6. Calvin H. I., Cooper G. W., Wallace E. W.: Evidence that selenium in rat sperm is associated with a cysteine-rich structural protein of the mitochondrial capsule. Gamete Res. 4, 139-149, 1981.
7. Chavez E. R., Patton J.: Response to injectable selenium and vitaminum E on reproductive performance of sows receiving a standard commercial diet. Can. J. Anim. Sci. 66, 1065-1068, 1986.
8. Dembinski Z., Bronicki M., Wandurski A.: Wplyw podawania selenu na rozrod swin. Med. Wet. 48 (4), 164-166, 1992.
9. Grela E., Baranowska M.: Witamina E i selen w zywieniu swin. Przeglad Hod. 5, 22-23, 1993.
10. Heimann E.D., Smith M.f., Morris J.S., Gall T.J., Elmore R.G., Morrow R.E.: Relationships among spermatozoal anormalities and the selenium concentration of blood plasma, semen, and reproductive tissues in young bulls.Anim. Reprod. Sci. 7, 315-321, 1984.
11. Jaskowski J. M.: Wplyw selenu podawanego w roznych terminach przed wycieleniem na przebieg okresu poporodowego i plodnosc krow. Med. Wet. 46 (7), 247-250, 1990.
12. Julien W. E., Conrad H. R., Moron A. L.: Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. J. Dairy Sci. 59 (11), 1960-1962, 1976.
13. Kleene K.C.: The Mitochondrial Capsule Selenoprotein - A Structural Protein in the Mitochondrial Capsule of Mammalian Sperm, in: Selenium in Biology and Human Health, ed. R.F. Burk, Springer-Verlag, New York 7, 135-149, 1993.
14. Mann T.: Ostatnie osiagniecia naukowe w dziedzinie biochemii nasienia i ukladu rozrodczego meskiego. Zesz. Nauk. AR Krakow 1974.
15. Pallini V., Bacci E.: Bull sperm selenium is bound to a structural protein of mitochondria. J. Submicr. Cytol. 11, 165-170, 1979.
16. Peretz A., Neve J., Duchateau J., Siderova V., Huygen K., Famaey J. P., Carpentier Y. A.: Effects of selenium supplementation on immune parameters in gut failure patients on home parenteral nutrition. Nutrition 7, 215-221, 1991.
17. Ramisz A., Buzek T.: Badania nad wplywem preparatu Evetsel na plennosc u owiec. Now. Wet. 3-4, 225-228, 1986.
18. Saake R. G., White J. M.: Semen quality testsand their relationship to fertility. Proc. Fourth Techn. conf. Anim. Reprod. and Artif. Insem. 2, 1, 1974.
19. Saaranen M., Suisoma U., Vanha-Perttula T.: Semen selenium content and sperm mitochomdrial volume in human and some animal species. Human Reprod. 4 (3), 304-308, 1989.
20. Siegel R.B., Murray F. A., Conrad H. R., Moxon A. L.: Effects on in vitro selenium on bovine sperm motility. Therio genology 13, 360-367, 1980.
21. Strzezek J.: Struktury morfologiczne plemnika i ich funkcja biochemiczna. Biul. Inf. ART Olsztyn, 25, 67, 1988.
22. Trinder N., Woodhouse C.D., Renton C.P.: The effect of vitamin E and Se on the retained placenta in dairy cows. Vet. Rec. 85, 550-553, 1969.
23. Udala J., Ramisz A., Drewnowski W., Lasota B., Radoch W.: Jakosc nasienia po podaniu buhajom selenu i witaminy E. Zeszyty Nauk. AR Szczecin 168, 57-63, 1995.
24. Wallace E., Calvin H. I., Cooper G. W.: Progressive effects observed in mouse sperm during course of three generations of selenium deficiency. Gamete Res. 4, 377-387, 1983.

25. Wierzbowski S., Kareta W.: Badanie przydatnosci rozplodowej trykow. Instytut Zootechniki, Krakow 1983.
26. Wu S. H., Oldfield J. E., Shull L. R., Cheeke P. R.: Specific effect of selenium deficiency on rat sperm. Biol. Reprod. 20, 793-798, 1979.
27. Zachara B. A., Mikolajczak J., Trafikowska U.: Effect of Various Dietary Selenium (Se) Intakes on Tissue Se Levels and Glutathione Peroxidase Actities in Lambs. J. Vet. Med. A. 40, 310-318, 1993.
28. Zachara B. A., Trafikowska U., Labedzka H., Mikolajczak J.: Effect of dietary Se intake on blood Se levels and glutathione peroxidase activities in lambs. Small Ruminant Res. 9, 331-340, 1993.

Submited:

Beata Seremak, Jan Udala, Bogdan Lasota
Department of Animal Reproduction
Agricultural University, Szczecin, Poland
ul. Judyma 6, 71-466 SZCZECIN, Poland
tel: +48 91 45-41-521, extension number 333