

## THE EFFECT OF INJECTIVE APPLICATION OF SELENOPIRAN ON THE PROLONGED INCREASE OF THE SELENIUM CONTENT IN BLOOD AND SPERM OF RAMS<sup>1</sup>

**E. Kistanova<sup>2</sup>, N. Metodiev<sup>3</sup>, E. Raycheva<sup>3</sup>, D. Abadjieva<sup>2</sup>, R. Stefanov<sup>2</sup>, V. Mladenova<sup>2</sup>, B. Blazhev<sup>4</sup>, M. Nevitov<sup>5</sup>, G. Boryaev<sup>5</sup>**

<sup>2</sup>Institute of biology and immunology of reproduction, BAS, Sofia, Bulgaria

<sup>3</sup>Institute of animal sciences, Kostinbrod, Bulgaria

<sup>4</sup>Central Laboratory for Chemical Testing and Control, BFSa, Sofia, Bulgaria

<sup>5</sup>Penza state agricultural academy, Penza, Russia

Corresponding author: kistanova@gmail.com

Original scientific paper

**Abstract:** Selenium is a microelement of big importance for male reproduction. As a part of the antioxidative enzyme glutathionperoxidase and structural Se proteins, it plays pivotal role in the defense of spermatozoa against generated ROS and in ensuring of its motility. During the last years the interests to the organic forms of selenium was enhanced because of its better biological utilization. The present work aimed to study the effect of injective application of organic compound selenopyran in rams on the distribution of selenium content in blood and sperm and on the changes in sperm quality. The experiment was conducted with 5 rams from the Synthetic Population Bulgarian Milk breed at the age between 3-7 years and live weight 85-90 kg. The animals were injected once with an oil solution of the selenopyran in dose of 0.1mg/kg live weight (selenium content 24%) 45 days before starting the breeding season. The blood was collected before treatment and 45 days thereafter. At the same time the first and second ejaculates of rams were collected using artificial vagina and analyzed by Sperm Class Analyzer. The selenium content was measured in plasma, blood cells and sperm by atomic absorption spectrometry using SpectrAA 55B double beam spectrometer (Varian, Inc.). The results showed that one injection of organic compound of selenium – selenopyran in dose of 0.1mg/kg live weight ensured the increase and support of high level of selenium in blood (plasma and blood cells) and in sperm during investigated period. That lead to the proper spermatogenesis in

---

<sup>1</sup> The paper presented at the 4th International Congress „New Perspectives and Challenges of Sustainable Livestock Production“, Belgrade, Serbia, 7th – 9th October, 2015

testis and allowed the production of qualitative ejaculates with higher number of total spermatozoa as well as of spermatozoa with progressive motility.

**Key words:** rams, Synthetic Population Bulgarian Milk sheep breed, selenium, spermatogenesis, selenopyran, injective application.

## Introduction

During the last decades there are new evidences of selenium importance for the male reproduction, especially for the properly spermatogenesis. More than 25 selenoproteins were identified in the live organisms. Most part of them occurs in the male reproductive system at tissue (testis, epididymal epithelium), cellular (intracellular membranes) and subcellular level (sperm nucleus, mitochondrial capsule) (*Ahsan et al., 2014*). Se concentrations in rodent testes exceed that of other organs, except kidneys (*Schriever et al., 2009*). According to *Kehr et al. (2009)* the distribution of Se in midpiece and head of spermatozoa is 4:1. Spermatozoa may be more vulnerable to oxidative stress if the Se content in selenoproteins is low and likely decreases the possibility of fertilization (*Beckett and Arthur, 2005*).

Also many investigations had underlined that selenium is essential for normal spermatozoa development and function in livestock animals (*Shi et al., 2010; Kendall et al., 2000; Tareq et al., 2010*). Se is actively incorporated into the developing spermatozoa of various mammalian species, including rats (*Burk et al., 1972*), bulls (*Bartle et al., 1980*) and rams (*Pond et al., 1983*).

The polygamy of the rams and short breeding season requires good condition for semen production. For the achievement of the optimal reproductive performances during the breeding season, where rams are used intensively, their diets require an additional feed additives, including selenium at first. However, there is a lack of information for a definition of an optimal Se status in blood and sperm of rams with regard to fertility.

Despite the many studies on the effects of selenium during spermatogenesis, more detailed investigations are required in order to provide a more clear understanding about relationship between selenium content in blood and in sperm.

The aims of the present study were to examine: a) effect of injective application of organic compound selenopyran on the Se content in blood and sperm of rams; b) the correlation between the Se content in blood and sperm and sperm quality during the preparation of rams to breeding season.

## Material and methods

The experiment was carried out with 5 rams from Synthetic Population Bulgarian Milk sheep breed, housed at Animal facility of the Institute of Animal Sciences - Kostinbrod. This breed is newly created (officially acknowledged during 2005) and it is the most spread breed in Bulgaria now. Rams were at the age - between 3 and 7 years old with live weight of 80 to 95 kg. The animals were raised in pens and fed with meadow hay *ad libitum* and concentrated mix forage 500 g/head (250 g wheat and 250 g Dried Distillers Grains with Solubles). Salt and mineral licks were placed in pens (EuroLick MultiVit<sup>®</sup>), as the concentration of Se in licks was 10 mg per kg. 45 days before breeding season the experimental rams were injected once subcutaneously with oil solution of selenopyran (9-phenyl-symmetrical octahydro-selenoxanthene) in dose of 0.1 mg /kg live weight. The Se content in this organic source was 24%. As mentioned in previous investigations (*Abadjieva et al., 2014*), the advantages of selenopyran are the lower toxicity in comparison with sodium selenite ( $LD_{50}=1600$  mg/kg against  $LD_{50}=3.25$  mg/kg) and ability to slowly liberate the selenium according to the needs of the organisms (*Boryaev and Kravchenko, 2006*).

### Blood collection

The blood samples were collected before treatment and 45 days thereafter. The blood was collected from *v. jagualris* in vacutainers covered with EDTA. Plasma and blood cells were separated by centrifuge at 3000 rpm for 15 min and stored at -20°C until analysis.

### Semen collection and analysis

From each ram the first and second ejaculates were collected by using artificial vagina in triplication (n=57 in total) before treatment and 45 days thereafter. At the time of sampling, the ejaculates were diluted (1:3, vol/vol) with 6A ram semen extender and transported to the IBIR-BAS laboratory within 1 hour. The estimation of semen quality parameters was done by Sperm Class Analyzer (SCA, Microptic, Spain) after appropriate additional dilution of samples. The total concentration, average percentage and number of motile sperm were measured by SCA software.

### Se measurement

The content of selenium was analyzed in sperm, in blood plasma and blood cells by the atomic absorption spectroscopy method in the Central Laboratory for Chemical Testing and Control –Bulgarian Food Safety Agency. All samples were digested using microwave pressure digestion system MARSXpress (CEM) with IR temperature sensor control and XP-1500 Plus fluoropolymer closed vessels. 0.5g sample with 5ml concentrate  $HNO_3$  was placed in vessels and heated to 185°C for

15min. The used reagents were of analytical reagent grade (Merck CGaA). For analysis stock solution of Se containing 1000 mg/ L (LGC Standards) was used for daily prepared analytical calibration standard with concentration 10µg/L by serial dilutions with 0.5% (v/v) HNO<sub>3</sub>. SpectrAA 55B double beam atomic absorption spectrometer (Varian, Inc.) was used for all determinations. Hollow cathode lamp from Varian operated at 10mA with spectral bandwidth of 1.0nm. The selected wavelength was 196.0 nm. Argon (99.996% purity) was used for carrier gas.

The statistical processing of the data was done by the STATISTIC computer programme (Stat Soft Inc., Ver.10.0). The one-way and regression analysis were done. The mean differences considered statistically significant at P<0.05.

## Results and discussion

The distribution of Se in ram blood plasma, blood cells and sperm are presented in the Table 1.

**Table 1. Content of selenium in blood and sperm of the treated rams**

Parameters	Se content, µg/L											
	Blood plasma				Blood cells				Sperm			
Rams (n=5)	Mean	SEM	Min	Max	Mean	SEM	Min	Max	Mean	SEM	Min	Max
Before treatment	59.3	14.9	25.3	106.5	<20	-	-	-	143.3	20.1	124.3	174.0
45 days thereafter	2026.3	219.7	1423.2	2581.0	654.7	87.0	472.2	943.4	637.3	66.0	509.4	883.6
P	0.000020				0.001				0.000385			

The blood Se concentration measured before treatment showed that the animals should be considered as a Se deficiently because levels below 70 µg/L are subnormal (*Pavlata et al., 2000*). The injective application of selenopyran leads to significant increase of the Se level in both plasma and blood cells.

Most literature data presents either serum or whole blood Se concentration analyses. Serum Se concentration reflects more acute or recent changes in Se nutrition or injective input of Se, whereas whole blood Se reflects more chronic or "historical" Se status (*Stowe and Herdt, 1992*). In our study we investigated blood plasma and blood cells separately and established that Se content in plasma was about 3 folders higher than in blood cells in both before treatment and 45 days thereafter.

These results should be explained trough the injective application of selenopyran and its immediate introduction to blood plasma. The majority of the glutathione peroxidase contented Se is incorporated into the red blood cells at the

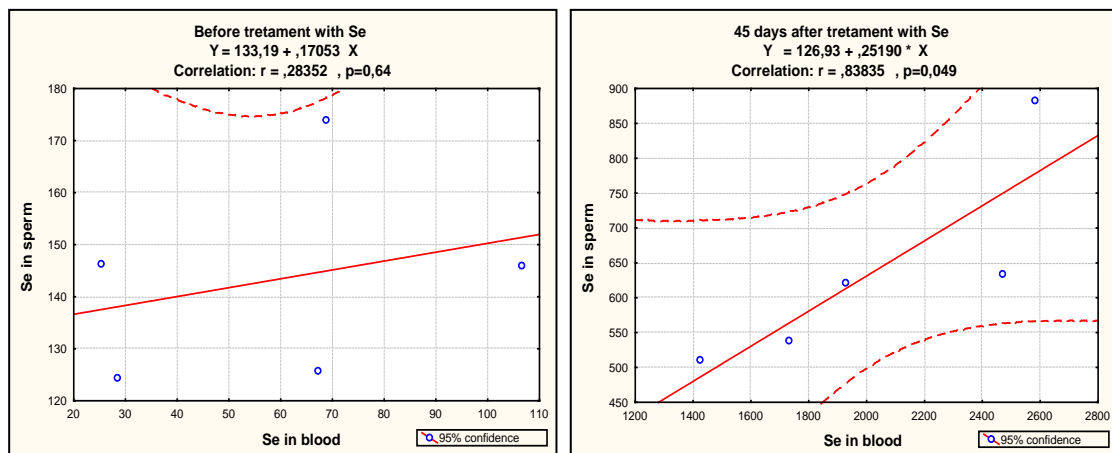
time of erythropoiesis and the response to a Se treatment in blood cells requires a time. That corresponds with our results: the high level of Se appears in blood cells only 45 days after treatment.

Compared to the reference range (*Stowe and Herdt, 1992*) of selenium in blood serum 120–150 µg/L for ewes, the average values in our rams, injected with 0.1mg/kg live weight selenopyran, were very high. Despite such high level of Se in blood we didn't observe any toxic effects. We suppose that the most part of Se in blood plasma is presented in the form of selenopyran and slowly released Se. Moreover, the quality of sperm after treatment with selenopyran was improved (Table 2). The level of Se in sperm significantly increased and remained high till 45-th day after treatment. Also the close correlation between Se level in blood plasma and sperm was established after treatment with selenopyran (Fig.1,  $r=0.84$ ;  $p=0.049$ ). These results confirmed low toxicity of preparation of organic compound selenopyran by its unique chemical structure where selenium atom is binding to the heterocyclic ring (*Boryaev and Kravchenko, 2006*). The similar results about low toxicity of organic selenium were reported in cows: the treatment with selenium yeast lead to high concentration of Se in blood (more than 1000 µg/L) without negative effects (*Juniper et al, 2008*).

Whole blood Se is an indicator of circulating Se and it reflects Se status. After treatment we found the largest amount of Se in blood plasma, followed by blood cells and whole semen. *Cheah and Yang (2011)* underline that sperm count and concentration of selenium in semen are in direct ratio. The results of our experiment confirm this statement (Table 2): the concentration of spermatozoa increased with the increase of the Se content in semen. Also the number of spermatozoa with progressive motility was enhanced after selenopyran treatment.

**Table 2. Sperm characteristic of investigated rams**

Rams( n=5)	Concentration of spermatozoa, mln/ml		Number of spermatozoa with progressive motility, mln/ml		% of progressive motility		Volume, ml	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<b>Before treatment, (n=28)</b>	849.3	56.6	117.6	10.6	14.4	1.2	0.86	0.06
<b>45 days thereafter, (n=29)</b>	1423.1*	135.6	209.4*	28.3	13.7	1.1	1.0	0.08
<b>P</b>	0.00031		0.00407		0.68604		0.34734	



**Figure 1. Correlation between selenium content in blood and sperm**

The mechanism of Se metabolism regulation is strongly dependent on species. In men, *Iwanier and Zachara (1995)* found similar to ours relations in Se content in blood and sperm. In bulls, the Se content was 10 times higher in seminal plasma than in blood serum (*Saaranen et al., 1989*).

Our results demonstrate the important role of Se for ram reproduction showing significant increase of Se content in sperm before breeding season, when spermatogenesis initiates. The high Se concentration during spermatogenesis is related to its protective property and its associated enzymes, such as mitochondrial capsule protein in spermatozoa (*Alabi et al., 2000; Kehr et al., 2009*). The increase of Se content in sperm in our experiment probably ensures the sufficient amount of Se to be taken into the spermatozoa.

## Conclusion

The obtained results showed that one injection of organic compound of selenium – selenopyran in dose of 0.1mg/kg live weight supports a high level of selenium in blood (plasma and blood cells) during the 45 days (spermatogenesis period in rams) and provides the sufficient amount of Se in sperm that ensures the quality ejaculates with higher number of total spermatozoa, as well as of spermatozoa with progressive motility.

## Acknowledgment

The research and participation in the 4 International Congress "New perspectives and challenges of sustainable livestock production" (Belgrade, Serbia, 7-9 October, 2015) is supported by grants №BG051PO001-3.3.06-0059 and №DKOF7RP02/17 under Ministry of Education and Science of Bulgaria and the European Social Fund and Operational Programme Human Resources Development.

## Uticaj injektivne primene selenopirana na produženo povećanje sadržaja selena u krvi i spermi ovnova

*E. Kistanova, N. Metodiev, E. Raycheva, D. Abadjieva, R. Stefanov, V. Mladenova, B. Blazhev, M. Nevitov, G. Boryaev*

## Rezime

Selen je mikroelement od velikog značaja za mušku reprodukciju. Kao deo antioksidativnog enzima glutationperokidaze i strukturnih Se proteina, on igra ključnu ulogu u odbrani i obezbeđivanju pokretljivosti spermatozoida. Tokom poslednjih godina zainteresovanost za organske oblike selena je povećana zbog njegove bolje biološke iskoristivosti. Ovaj rad ima za cilj da prouči efekat injektivne primene organskog jedinjenja selenopiran u ovnova na distribuciju i sadržaj selena u krvi i spermi i na promene u kvalitetu sperme. Ogled je izveden sa 5 ovnova sintetičke populacije bugarske mlečne rase u uzrastu od 3-7 godina i telesne mase 85-90 kg. Životinjama je jednom ubrizgavan uljni rastvor selenopiran u dozi od 0.1 mg/kg žive težine (sadržaj selena 24%) 45 dana pre početka sezone parenja. Krv je sakupljena pre tretmana i 45 dana nakon toga. Istovremeno, prvi i drugi ejakulat ovnova je uzet pomoću veštačke vagine i analiziran pomoću Sperm Class Analyzer. Sadržaj selena je meren u plazmi, krvnim ćelijama i spermi atomskom apsorpcionom spektrometrijom korišćenjem SpectrAA 55B double beam spectrometer (Varian, Inc.) - spektrometar dvostrukog snopa.

Rezultati su pokazali da jedna injekcija organskog jedinjenja selena – selenopirana, u dozi od 0,1 mg/kg žive mase osigurava povećanje i održavanje visokog nivoa selena u krvi (plazma i krvne ćelije) i u spermi tokom perioda ispitivanja. To je dovelo do odgovarajuće spermatogeneze u testisima i omogućilo je proizvodnju kvalitetnih ejakulata sa većim brojem ukupnih spermatozoida, kao i spermatozoida sa progresivnim pokretljivošću.

## References

- ABADJIEVA V.D., KISTANOVA E., MARCHEV Y., NEDEVA R., VAISBERG C., STEFANOV GEORGIEV R., BORYAEV G., NEVITOV M. (2014): Improvement of the antioxidative status of pig ovaries by selenopyran treatment. *Mac Vet Rev*, 37, 2, 165-170.
- AHSAN U., KAMRAN Z., RAZA I., AHMAD S., BABAR W., RIAZ H.M., IQBAL Z. (2014): Role of selenium in male reproduction—A review. *Animal Reproduction Science*, 146, 55–62 .
- ALABI S.N., BEILSTEIN A.M., WHANGER D.P. (2000): Chemical Forms of Selenium Present in Rat and Ram Spermatozoa In Vivo and In Vitro Studies. *Biological Trace Element Research*, 76, 161-172.
- BARTLE J. L., SENGER L.P., HILLERS K.J. (1980): Influence of injected selenium in dairy bulls on blood and semen selenium, glutathione peroxidase and seminal quality, *Biol. Reprod.*, 23, 1007–1013.
- BECKETT G.J., ARTHUR J.R. (2005): Selenium and endocrine systems. *Journal of Endocrinology*, 84, 455-465.
- BORYAEV G.I., KRAVCHENKO YU.V. (2006): Selenopyran is organic compound of selenium with original biological properties. International congress Euromedica, Hannover, Germany, p15-16. <http://www.congress-euromedica.de/abstrakt/broschuere2006.pdf>
- BURK F., BROWN G.D., SEALY J.R., SCAIEF C.C. (1972): Influence of dietary and injected selenium on while-body retention, route excretion and tissue retention of  $^{75}\text{SeO}_3^{2-}$  in the rat. *J. Nutr.*, 102, 1049–1056.
- CHEAH Y., YANG W. (2011): Functions of essential nutrition for high quality spermatogenesis. *Advances in Bioscience and Biotechnology*, 2, 182-197.
- IWANIER K., ZACHARA BA. (1995): Selenium supplementation enhances the element concentration in blood and seminal fluid but does not change the spermatozoal quality characteristics in subfertile men. *J Androl.*, 16, 5, 441-7.
- JUNIPER D.T., PHIPPS R.H., GIVENS D.I., JONES A.K., GREEN C., BERTIN G. (2008): Tolerance of ruminant animals to high dose in-feed administration of a selenium-enriched yeast. *J Anim Sci* , 86, 1, 197-204.
- KEHR S., MALINOUSKI M., FINNEY L., VOGT S., LABUNSKYY V.M., KASAIKINA M.V., CARLSON B.A., ZHOU Y., HATFIELD, D.L., GLADYSHEV, V.N. (2009): X-rayfluorescence microscopy reveals the role of selenium in spermatogenesis. *J. Mol. Biol.*, 389, 808–818.
- KENDALL N. R., MCMLLEN G., REEN S.A., RODWAY R.G. (2000): The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. *Anim Rep Sci*, 62, 4, 277-83.
- PAVLATA L., PECHOVA. A., ILLEK J. (2000): Direct and indirect assessment of selenium status in cattle – a comparison. *Acta Vet Brno*, 69, 281-287.



- 
- POND F.R., TRIPP J.M., WU H.S.A., WHANGER D.P., CHMITZ A.J. (1983): Incorporation of selenium-75 into semen and reproductive tissues of bulls and rams. *J. Reprod. Fertil.*, 69, 411–418.
- SAARANEN M., SUISTOMAA U., VANHA-PERTTULA T. (1989): Semen selenium content and sperm mitochondrial volume in human and some animal species. *Hum Reprod.*, 4, 3, 304-8.
- SCHRIEVER S.C., BARNES K.M., EVENSON J.K., RAINES A.M., SUNDE R.A. (2009): Selenium requirements are higher for glutathione peroxidase-1 mRNA than gpx1 ac-tivity in rat testis. *Experimental Biology and Medicine* (Maywood), 234, 513-521.
- SHI L., ZHANG C., YUE W., SHI L., ZHU X., LEI F. (2010): Short term effect of dietary selenium–enriched yeast on semen parameters, antioxidant parameters and Se concentration in goat seminal plasma. *Anim Feed Sci Tech*, 157, 104-8.
- STOWE H.D., HERDT T.H. (1992): Clinical-assessment of selenium status of livestock. *Journal of Animal Science*, 70, 3928–3933.
- TAREQ K.M.A., MIAH A.G., SALMA U., YOSHIDA M., TSUJII H. (2010) Effect of selenium and vitamin E on acrosome reaction in porcine spermatozoa. *Reproduction of Medicine Biology*, 9, 73-81.

Received 28 May 2015; accepted for publication 2 August 2015