

UDC 636.4.082.31:577.122

POLISHCHUK S., PhD student

TSEKHMISTRENKO S., Dr. of agricultural Science

POLISHCHUK V., TSEKHMISTRENKO O., candidates of agricultural Science

SLUSARENKO A., candidates of veterinary Science

*Bila Tserkva National Agrarian University*

## **BREED FEATURES OF PROTEIN METABOLISM IN THE BOAR-SIRES ORGANISM**

The data of proteins oxidative modification intensity in semen plasma and spermocyte plasma of Large White breed boar-sires and synthetic line SS23 are being studied. It is determined that the contents of the products of oxidative modification of sperm plasma of both animal groups under study is much higher compared to spermocyte plasma. The pattern in the distribution and content of oxidized forms of proteins in the Large White breed boar-sires and synthetic line SS23 is noted, due to the intensity of metabolic processes. Investigated semen of boar-sires contained a significant amount of sulfhydryl groups of proteins and low molecular weight thiols

**Key words:** boar-sires, semen, the oxidative modification of proteins, the average weight molecules, thiol groups.

**Statement of the problem, analysis of recent research and publications.** Oxidative stress and its impact on the functioning of various body systems is an important problem for modern biology and medicine. Stress is seen as a way to achieve resistance to the action of extreme factors of different origin. However, stress can be a factor that promotes the development of several diseases [4, 6].

An important manifestation of the stress response and the adaptive adjustment is to improve the activity of the regulatory mechanisms involved in maintaining an optimal level of metabolic processes intensity at the level of the whole organism. However, there must be natural specific features in the implementation of various mechanisms for mobilizing stress. The problem of stress response implementation at the level of individual organs and tissues remains valid. In particular, there is little knowledge in question of metabolic processes changes in the boar-sires body under their moderate use.

Analysis of modern scientific literature leads to the conclusion that many papers are devoted to lipid peroxidation and at the same time the oxidative modification of (OMP) cells and tissues proteins are described less. Mechanisms and consequences of the stress response in animals depend of the different tissue metabolic capabilities and individual physiological capabilities.

**The aim of the study** is to determine the content of the oxidative modification of protein products and middle mass molecules in the boars semen, which is an important marker for the morphological and functional state of spermatozoa evaluation.

**Materials and methods.** The Large White breed boar-sires and synthetic line SS23 two year old ones were used for the research. Animals were raised in the conditions of the "Elite" Ltd. Company at Terezine village, Bila Tserkva district, Kyiv region. Stockkeeping conditions corresponded the general biological requirements.

The material for the study was the sperm, received by manual way. Sperm plasma was separated by centrifugation (3000 rev./min. during 10 minutes.), germ cells were washed in saline. Next freezing and thawing destroyed the sperm cell membrane, the final destruction was carried out by differential centrifugation at 14,000 rev/min. during 10 min. Then metabolic proteins products (carbonyl derivatives, the average molecular weight, thiol groups) were determined in semen plasma and spermocyte plasma.

OMP intensity was determined by reaction of protein amino acid oxidized residues with 2.4 dinitrophenylhydrazine to form aldehydinitrophenylhydrazones (ADNPH) and ketonedinitrophenylhydrazones (KDNPH). Absorbance of formed carbonyl derivatives dinitrophenylhydrazons is recorded at different wavelengths: 356 nm – KDNFH aliphatic neutral (KDNFH NH); 370 nm – ADNPH aliphatic neutral (ADNPH NH); 430 nm – aliphatic KDNFH main character (KDNFH OX); 530 nm – aliphatic ADNPH main character (ADNPH TC) [3].

Determination of average molecular mass (AMM) was carried out by screening method [5], which is based on the deposition of coarse fibers threechloroacetic acid 10% solution of acid. Optical density of the supernatant recorded at 254 nm – the average molecular weight that do not contain aromatic acids; 280 nm – the average molecular weight containing aromatic acids.

Determination of HS-groups of proteins [1] was performed using Ellman's reagent, which is based on the interaction of 5,5'-ditiobis-2-nitrobenzoic acid with HS-groups of proteins and releasing tionitrofenile anion. Tionitrofenile anion number's formed directly proportional to the content of HS-groups of proteins. The results are analyzed using packet processing applications for medical and biological information Statistica 6.0 (StatSoft, Inc., USA).

**Results and discussion.** OMP's datas of boar-sires plasma and semen spermotsyte plazma of Large White breed and synthetic line SS23 presents in table 1. The main dynitrofenilhidrazones content were formed belong to the aldehyde and ketonedynitrofenilhidrazones neutral. Significant difference between these parameters in the studies were not found.

Table 1 – Content of the oxidative modification of proteins products in semen plasma and spermocyte plasma of boar-sires, mmol/g protein, (M ± m; n = 8)

Group of animals		Products neutral		Products main	
		KDNPH λ=356	ADNFH λ=370	KDNPH λ=430	ADNFH λ=530
Purebred boar-sires of Large White breed	Plasma	40,21±2,44	31,29±1,72	25,36±1,80	3,50±0,30
	Spermocyte plasma	4,55±0,38	3,60±0,27	2,08±0,15	0,71±0,06
Boar-sires of synthetic line SS23	Plasma	34,23±2,29	32,72±2,12	24,74±1,49	4,3±0,40
	Spermocyte plasma	5,23±0,23	3,71±0,18	2,17±0,14	0,80±0,07

The neutral and main KDNFH content of the purebred boar-sires sperm plasma is slightly higher than in the group of synthetic line animals. Instead the number of investigated products in spermotsyte plasma of the Large White breed sires was lower compared with synthetic line animals.

The study of main and neutral ADNFH showed lower content of oxidation products in plasma and spermotsyte plasma in purebred animals. The concentration of OMP products in semen plasma of both animal groups is significantly higher compared to spermotsyte plasma.

Thus it is marked a certain regularity in the distribution and content of oxidized forms of proteins in the Large White breed boar-sires and synthetic line SS23. The OMP products level in the semen of studied animals was probably due to the different intensity of metabolic processes.

It was found that proteins semen of synthetic lines boar-sires are more susceptible to OMP. In the literature, there is evidence that the oxidation of proteins leads to degradation of the corresponding peptides [8]. It is known, that low molecular weight compounds peptides play an important role in the regulation of metabolic processes, especially under stress exposure [6].

Average molecule weight range in general is an indicator that reflects the destructive oxidative processes in the body. They are formed as a result of proteolysis and they contribute increasing of free radical processes in the body. Average weight molecules are markers of endogenous intoxication [2], as they regulate the flow of pathological processes and they take the role of secondary toxins with viability affect for all organs and systems. The average molecule weight level of spermocyte plasma of large white breed sires was significantly higher (p <0.05) compared with synthetic line animals SS23 (Table 2), which are containing aromatic amino acids.

Table 2 – The average weight molecules content in semen plasma and spermocyte plasma of boar-sires, conv. units. (M ± m; n = 8)

Index	Purebred boar-sires Large White breed		Synthetic line Boar-sires SS23	
	Plasma	Spermocyte plasma	Plasma	Spermocyte plasma
AWM with aromatic amine acids	0,17±0,01	0,21±0,01	0,15±0,01	0,16±0,01*
AWM without aromatic amine acids	0,82±0,04	1,05±0,05	0,73±0,04	0,86±0,05*

**Note.** Here and in Table. 3 significant difference is relatively to purebred boars-sires at \* – P <0.05.

Important role in the regulation of redox homeostasis restored in cells and tissues play a thiol compounds. HS-containing proteins are the main line of defense cells against radical OH •. Due to short half-life period and diffusion radius the hydroxyl radicals is not subject to enzymatic inactivation in biological systems. But these compounds exhibit strong cytotoxic and mutagenic effects [4, 7].

Male sex cells are rich for thiols as they play a crucial role in the functioning of the cell. The content of sulfhydryl groups can be seen on the enzymes metabolic activity. Thiol groups protect proteins from damaging by unfavorable external environment. So it is likely that the physiological activity of the

semen collected from boar-sires of different breeds is in direct proportion to the quantitative content of HS-groups in sperm.

It is established that semen plasma and spermocyte plasma of purebred boar-sires on average contained  $140,40 \pm 5,22$  and  $330,57 \pm 9,63$  mmol / L HS-groups, in synthetic line boar-sires –  $179,85 \pm 8,09$  and  $357,01 \pm 13,40$  mg / l HS-groups, respectively (Table 3).

Table 3 – Content of sulfhydryl groups in semen plasma and spermocyte plasma of boar-sires, (mmol / l, M  $\pm$  m; n = 8)

Index	Purebred boar-sires Large White breed		Boar-sires Synthetic line SS23	
	Plasma	Spermocyte plasma	Plasma	Spermocyte plasma
Common HS-group	140,40 $\pm$ 5,22	330,57 $\pm$ 9,63	179,85 $\pm$ 8,09*	357,01 $\pm$ 13,40
Free HS-group	27,90 $\pm$ 1,42	57,03 $\pm$ 3,01	33,23 $\pm$ 1,59*	81,60 $\pm$ 3,14*
Protein HS-group	112,50 $\pm$ 3,92	273,50 $\pm$ 14,14	146,62 $\pm$ 9,42*	275,40 $\pm$ 14,52

It is established that semen plasma and spermocyte plasma of purebred boar-sires on average contained  $140,40 \pm 5,22$  and  $330,57 \pm 9,63$  mmol / L HS-groups. The synthetic line boar-sires contained  $179,85 \pm 8,09$  and  $357,01 \pm 13,40$  mg / l HS-groups, respectively (Table 3).

The concentration of HS-groups in the boar-sires germ cells of both breeds was actually two times higher than in semen plasma. This indicates that sulfhydryl groups play important role in the regulation of gametes oxidation-reduction homeostasis. The number of common, free and protein sulfhydryl groups in the semen of synthetic lines boars was significantly higher in relation to animals of large white breed.

The protein thiol groups were dominated in spermocyte plasma and semen plasma of animals studied. Value of protein sulfhydryl groups to free (non-protein) sulfhydryl groups in semen plasma and spermocyte plasma of purebred sires is 4.03 and 4.79 units, in animals of synthetic line – 4.41 and 3.37 respectively. This suggests that spermocyte plasma of boar-sires has many more non-protein thiol groups.

**Conclusions.** Analysing the above data we can say that the boar-sires semen contains large amounts of sulfhydryl groups of low weight molecular thiols and proteins. These compounds could likely take active part in the complex of biochemical processes in cells leaking and structural elements stored in the cell membrane of gametes and quality indicators of sperm.

**Prospects for further research.** The correction of free radical oxidation of proteins and lipids by using a multicomponent drug.

#### LIST OF REFERENCES

1. Веревкина И.В. Колориметрический метод определения SH-групп и –S–S–связей в белках при помощи 5,5'-дитиобис (2-нитробензойной) кислоты / И.В. Веревкина, А.И. Точилкин, Н.А. Попова // Современные методы в биохимии; под ред. В.Н. Ореховича. – М.: Медицина, 1977. – С. 223–228.
2. Горбатова Л.Н. Характер распределения веществ низкой и средней молекулярной массы в биологических жидкостях у практически здоровых детей / Л.Н. Горбатова, Н.А. Бебякова, А.В. Сумарокова // Физиология человека. – 2007. – Т. 41. – С. 114–118.
3. Дубинина Е.Е. Окислительная модификация белков сыворотки крови человека, метод ее определения / Е.Е. Дубинина, С.О. Бурмистров, Д.А. Ходов // Вопр. мед. химии. – 1995. – Т. 41. – С. 24–26.
4. Зенков Н.К. Окислительный стресс / Зенков Н.К., Ланкин В.З., Меньщикова Е.Б. – М.: Наука, 2001. – 343 с.
5. Скрининговый метод определения средних молекул в биологических жидкостях: Метод. рекомендации / Н.И. Габриэлян, Э.П. Левицкий, А.А. Дмитриев и др. // НИИТ и ИО МЗ СССР. – М., 1985. – 32 с.
6. Agarwal A. Effect of Oxidative Stress on Male Reproduction / A. Agarwal, G. Virk, C. Ong // World J. Mens Health. – 2014. – Vol. 32 (1). – P. 1–17.
7. Chen S.J. Influence of reactive oxygen species on human sperm functions and fertilizing capacity including therapeutical approaches / S.J. Chen, J.P. Allam, Y.G. Duan // Arch. Gynecol. Obstet. – 2013. – Vol. 288 (1). – P. 191–199.
8. Piomboni P. Protein modification as oxidative stress marker in normal and pathological human seminal plasma / P. Piomboni, A. Stendardi, L. Gambera // Redox Rep. – 2012. – Vol. 17 (5). – P. 227–232.

#### REFERENCES

1. Verevkyna Y.V. Kolorymetrychesky metod opredelenyya SH-hrupp y –S–S–svyazey v belkakh pry pomoshchy 5,5'-dytyobys (2-nitrobenzoynoy) kysloty / Y.V. Verevkyna, A.Y. Tochylkyn, N.A. Popova // Sovremennye metody v byokhymyy; pod red. V.N. Orekhovycha. – M.: Medytsyna, 1977. – S. 223–228.
2. Horbatova L.N. Kharakter raspredelenyya veshchestv nyzkoey y sredney molekulyarnoy massy v byolohycheskykh zhydkostyakh u praktychesky zdorovykh detey / L.N. Horbatova, N.A. Bebyakova, A.V. Sumarokova // Fyzyolohyya cheloveka. – 2007. – Т. 41. – S. 114–118.
3. Dubynyna E.E. Okyslytel'naya modyfykatsyya belkov syvorotky krovy cheloveka, metod ee opredelenyya / E.E. Dubynyna, S.O. Burmystrov, D.A. Khodov // Vopr. med. khymyy. – 1995. – Т. 41. – S. 24–26.
4. Zenkov N.K. Okyslytel'nyy stress / Zenkov N.K., Lankyn V.Z., Men'shchykova E.B. – M.: Nauka, 2001. – 343 s.

5. Skrynynhovyuy metod opredelenyya srednykh molekul v byolohycheskykh zhydkostyakh: Metod, rekomendatsyy / N.Y. Nabryelyan, E.R. Levvitsky, A.A. Dmytryev y dr. / NYIT y YO MZ SSSR. – M., 1985. – 32 s.

6. Agarwal A. Effect of Oxidative Stress on Male Reproduction / A. Agarwal, G. Virk, C. Ong // World J. Mens Health. – 2014. – Vol. 32 (1). – P. 1–17.

7. Chen S.J. Influence of reactive oxygen species on human sperm functions and fertilizing capacity including therapeutical approaches / S.J. Chen, J.P. Allam, Y.G. Duan // Arch. Gynecol. Obstet. – 2013. – Vol. 288 (1). – P. 191–199.

8. Piomboni P. Protein modification as oxidative stress marker in normal and pathological human seminal plasma / P. Piomboni, A. Stendardi, L. Gambera // Redox Rep. – 2012. – Vol. 17 (5). – P. 227–232.

#### **Породні особливості білкового обміну в організмі кнурів-плідників**

**С.А. Поліщук, С.І. Цехмістренко, В.М. Поліщук, О.С. Цехмістренко, А.О. Слюсаренко**

Наведені дані щодо дослідження інтенсивності окиснювальної модифікації білків у плазмі сперми та спермоцитоплазмі кнурів-плідників великої білої породи та синтетичної лінії SS23. Встановлено, що вміст продуктів окиснювальної модифікації в плазмі сперми обох досліджуваних тварин значно вищий порівняно із спермоцитоплазмою. Відмічена закономірність у розподілі та вмісті окиснених форм білків у кнурів-плідників великої білої породи та синтетичної лінії SS23, що обумовлено інтенсивністю метаболічних процесів. Досліджена сперма кнурів-плідників містила значну кількість сульфогідрильних груп низькомолекулярних тиолів і білків.

**Ключові слова:** кнури-плідники, сперма, окиснювальна модифікація білків, молекули середньої маси, тиолові групи.

#### **Породные особенности белкового обмена в организме хряков-производителей**

**С.А. Полищук, С.И. Цехмистренко, В.Н. Полищук, О.С. Цехмистренко, А.А. Слюсаренко**

Приведены данные по исследованию интенсивности окислительной модификации белков в плазме спермы и спермоцитоплазме хряков-производителей крупной белой породы и синтетической линии SS23. Установлено, что содержание продуктов окислительной модификации в плазме спермы обеих исследуемых животных значительно выше по сравнению с спермоцитоплазмой. Отмечена закономерность в распределении и содержании окисленных форм белков у хряков-производителей крупной белой породы и синтетической линии SS23, что обусловлено интенсивностью метаболических процессов. Исследована сперма хряков-производителей содержала значительное количество сульфгидрильных групп низкомолекулярных тиолов и белков.

**Ключевые слова:** хряки-производители, сперма, окислительная модификация белков, молекулы средней массы, тиоловые группы.

*Надійшла 9.10.2014.*