Matysik S.I., Kuzminov B.P., Ostapiv D.D. Cytotoxic action of hepatoprotector antral on bull sperm (на англ. яз.; *Матысик С.И., Кузьминов Б.П., Остапив Д.Д.* Цитотоксическое действие гепатопротектора антраля на сперму быков)

DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209
Оригинальная статья

Matysik S.I.[1], Kuzminov B.P.[1], Ostapiv D.D.[2]

Cytotoxic action of hepatoprotector Antral on bull sperm

[1]Danylo Halytsky Lviv National Medical University Department of hygiene and prophylactic toxicology, 79010, Lviv, Ukraine;
[2]Institute of animal biology of NAAS, Lviv, 79034, Ukraine

Introduction. *According to the European legislation and requirements, industrial production of medication shall be provided with well-defined regulations of the quality of such preparation.*
Material and methods. *Cytotoxic action of hepatoprotector Antral on bull sperm was evaluated. Samples of sperm were divided into control and two experimental samples: control one was diluted with phosphate-buffered saline (PSB), which included NaCl – 0.8 g, KCl – 0.02 g, Na_2HPO_4 – 0.11 g, KH_2PO_4 – 0.02 g, $MgCl_2$ – 0.01 г, H_2O till 100ml; in the first experimental sample PSB was added with antral in the dose 1/500 LD50 (2.77 mg); in the second – PSB with Antral in the dose 1/100 LD50 (13.87 mg) was added. The survival of spermatozoa was defined until termination of rectilinear forward movement in sperm, which was preserved in temperature of 2-5 °C; respiratory activity was defined (by polarography (ng-atom O/0.1 ml of semen (S) · min) in thermostated sell (temperature of 38.5 °C), with the volume of 1.0 ml with the automatic registration of process flow by potentiometer, the proportion of decrease of which was evaluated in accordance to impacted dose of antral in substrate and restorative activity was defined potentiometrically (mV/0.1ml C · min) using a system of pen microelectrodes that were inserted in thermostated polarographic cell).*
Results. *In the experimental samples of semen in comparison with the control sample the respiratory and restorative activities were reduced; dose 1/500 LD50 to 58.8% and dose 1/100 LD50 to 68.5%; restorative activity – to 66.7% and 54.5% correspondingly.*
Conclusion. *Antral reduces the survival of sperm being irrespective of dose.*

K e y w o r d s : Antral; cytotoxic action; bull sperm.

For citation: Matysik S.I., Kuzminov B.P., Ostapiv D.D. Cytotoxic action of hepatoprotector Antral on bull sperm. *Gigiena i Sanitaria (Hygiene and Sanitation, Russian journal).* 2020; 99(2): 206-209. (In Russ.). DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209

For correspondence: *Svetlana I. Matysik*, Assistant at the Department of hygiene and prophylactic toxicology of the Danylo Halytsky Lviv National Medical University, Lviv, 79010, Ukraine. E-mail: lana77matysik@gmail.com

Information about authors: Matysik S.I., https://orcid.org/0000-0002-2251-4948

Conflict of interest. The authors declare no conflict of interest.
Acknowledgment. The study had no sponsorship.
Contribution: Research concept and design – Ostapiv D.D.; Statistical polishing of the material – Matysik S.I.; Writing of the text – Matysik S.I., Kuzminov B.P.; Manuscript text editing – Kuzminov B.P.

Received: August 21, 2018
Accepted: December 12, 2019
Published: March 23, 2020

Матысик С.И.[1], Кузьминов Б.П.[1], Остапив Д.Д.[2]

Цитотоксическое действие гепатопротектора антраля на сперму быков

[1]Львовский национальный медицинский университет им. Данила Галицкого, 79010, Львов, Украина;
[2]Институт биологии животных НААН, 79034, Львов, Украина

Введение. *Согласно с Европейским законодательством и требованиями, промышленный выпуск препарата должен быть обеспечен чётко очерченным регламентом качества проведения такого вида производства, что обусловливает проведение обязательной токсикологической оценки.*
Материал и методы. *Проведена оценка цитотоксического влияния гепатопротектора антраля на спермиях быков. Пробы спермы делили на контрольную и две опытные: контрольную пробу разжижали фосфатно-солевым буфером (ФСБ), в состав которого входят NaCl – 0,8 г, KCl – 0,02 г, Na_2HPO_4 – 0,11 г, KH_2PO_4 – 0,02 г $MgCl_2$ – 0,01 г, H_2O – до 100 мл; в первую опытную пробу добавляли ФСБ с антралем в дозе 1/500 LD_{50} (2,77 мг), во вторую – ФСБ с антралем в дозе 1/100 LD_{50} (13,87 мг). В пробах определяли выживаемость спермиев (ч), которые хранились при температуре 2–5 °С, по времени прекращения их прямолинейно-поступательного движения; дыхательную активность (полярографически (нг-атом О/0,1 мл спермы (С) · мин) в термостатированной ячейке (температура 38,5 °С) объёмом 1 мл с автоматической регистрацией течения процесса потенциометром; пропорциональность, снижение которой оценивали в соотношении к введённой дозе антраля в субстрате), восстановительную активность (потенциометрически (mV/0,1 мл с · мин) с использованием системы открытых микроэлектродов, которые вставляли в термостатированную полярографическую ячейку.*

DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209
Original article

Результаты. *В опытных пробах по сравнению с контрольной уменьшилась как дыхательная, так и восстановительная активность спермы: доза 1/500 LD_{50} на 58,8% и доза 1/100 LD_{50} – на 68,5%; восстановительная активность – на 66,7 и 54,5% соответственно.*
Заключение. *Антраль снижает выживаемость сперматозоидов независимо от дозы.*

К л ю ч е в ы е с л о в а : антраль; цитотоксическое действие; спермии быков.

Для цитирования: Матысик С.И., Кузьминов Б.П., Остапив Д.Д. Цитотоксическое действие гепатопротектора антраля на сперму быков. *Гигиена и санитария.* 2020; 99 (2): 206-209. DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209

Для корреспонденции: *Матысик Светлана Игоревна*, ассист. каф. гигиены и профилактической токсикологии Львовского национального медицинского университета им. Данила Галицкого, 79010, Львов, Украина. E-mail: lana77matysik@gmail.com

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.
Финансирование. Исследование не имело спонсорской поддержки.
Участие авторов: концепция и дизайн исследования – Остапив Д.Д.; статистическая обработка материала – Матысик С.И.; написание текста – Матысик С.И., Кузьминов Б.П.; редактирование – Кузьминов Б.П.

Поступила: 21.08.18
Принята к печати: 12.12.19
Опубликована: 23.03.2020

Relevance

The influence of the environmental pollution factor, is burdened with the difficult economic situation, wide-spread harmful habits among population, popularization and unsubstantiated use of medications, biologically active substances, stimulants, useless food products, stipulates increase of the number of liver disease, which, as the main organ of detoxification, is affected first. According to the data of the WHO, the annual growth rate of liver disease reaches 2billion in the world, that requires the use of effective drugs with a selective action on a hepatobiliary system of a person [4]. One of such drugs in modern therapy is domestic hepatoprotector antral, which also has antiulcer and antioxidant effects. Use of this drug in the field of hepatology and gastroenterology is stipulated by its impact on the respiratory and restorative activity of cells, reducing oxygen consumption [13].

According to the European legislation and requirements [12], industrial production of medication shall be provided with well-defined regulations of the quality of such production, what stipulates conduction of compulsory toxicological assessment of the influence of antral (in this case) on the organisms of people working, with compulsory substantiation of the safe reference level of impact.

The present modern list of compulsory methods of the assessment of potential hazards of toxic substances on the health of population and environment stipulates for engagement of medication, highly-informative, non-invasive and express methods, which include study of the impact of medication on alternative test-systems - cell cultures Daphnia magnastraus, bull sperm, etc. [6,7]. Such methods enable to study specificity and the direction of the toxic influence of the active substance on the culture of cells from different organs and systems of the organism in isolation or in model environments, and to assess efficiency of the action of the medications in regards to their pharmacokinetics and pharmacodynamics in experiments short enough [2,5].Having high sensitivity to the influence of toxic substances and being easily standardized, bull sperm suspension is widely used in the practice of toxicological studies [1, 3, 5].

The purpose of the work was the assessment of the cytotoxic action of antral on bull sperm.

Material and methods

Antral is a synthetic hepatoprotector, which is an active pharmaceutical ingredient, containing tris-{N-(2,3-dimethylphenyl)anthranilate} aluminium - 0,1 or 0,2g. The medication is moderately soluble in chloroform, and practically insoluble in water, 96% ethyl alcohol, acetone and hexanes.The median lethal dose (LD_{50}) for white rates - 1387,5 mg/kg [27].

Cytotoxic action of tris-{N-(2,3-dimethylphenyl)anthranilate} aluminium in the form of the suspension was studied on fresh ejaculates of 4 bulls with the following physiological characteristics: sperm volume 4-5 ml, sperm cells concentration 0,7-1,2×10^9, cells/ml, number of live spermatozoa amounted to 70-85 %. Samples of sperm were divided into control and two experimental samples: control one was diluted with phosphate-buffered saline (PSB), which included NaCl - 0,8 г, KCl - 0,02 г, Na_2HPO_4 - 0,11 г, KH_2PO_4 - 0,02 g, $MgCl_2$ - 0,01 г, H_2O till 100ml, in the first experimental sample PSB was added with antral in the dose 1/500 LD50 (2,77 mg); in the second – PSB with antral in the dose 1/100 LD50 (13,87 mg) was added. The survival of spermatozoa was defined until termination of rectilinear forward movement in sperm, which was preserved in temperature 2-5 °C ; respiratory activity was defined (by polarography (ng-atom O/0,1 ml of semen (S) · min) in thermostated sell (temperature 38,5 °C), with the volume 1,0 ml with the automatic registration of process flow by potentiometer, the proportion of decrease of which was evaluated in accordance to impacted dose of antral in substrate and restorative activity was defined potentiometrically (mV/0,1ml C · min) using a system of pen microelectrodes that were inserted in thermostated polarographic cell [15,16,17]). Statistical processing of study results was held by M.O.Plokhinskyi [10].

Results

In the experimental samples of semen in comparison with the control sample the respiratory and restorative activities were reduced; dose 1/500 LD50 was accompanied by oxygen reduction to 58,8% ($p < 0,01$), and dose 1/100 LD_{50} to 68,5% ($p < 0,01$); restorative activity reduced to 66,7% ($p < 0,001$) and 54,5% ($p < 0,01$) accordingly. No reliable dose-dependent changes of the studied biochemical parameters were determined at exposure to different doses of the medication.

The influence of antral on the intensity of oxidation processes is confirmed by strong correlational dependence between the dose of the pharmacological drug and the value of respiratory and restorative activity ($\eta^2 = 0,76$ and 0,82 accordingly).

Reduction of the parameters of the oxidation processes in sperm under the influence of antral in dose 1/500 LD_{50}did not affect cell survival, which amounted to 102,0 ± 9,95 and was at the control level (table). Instead low respiratory and restorative activity of spermatozoa in the sample with hepatoprotector in dose 1/100 LD_{50}, probably, lead to reduction of their survival time, which was lower for 30,0 hours ((27,2 %; $p < 0,01$) compared to the control and for 24 hours (25,6 %; $p = 0,1$) compared to the action of the medication in dose 1/500 LD_{50}.

Correlation dependence between the influence of increasing doses of antral and survival of bull spermatozoa is weak ($\eta^2 = 0,438$), what may be explained by the sufficiently wide range between the therapeutic and median lethal dose of the medication, determined by us in experiments on laboratory animals [9].

Discussion

Respiratory activity of reproductive cells is related exclusively to the functioning of mitochondria, thus, oxygen consumption by spermatozoa is a feature of their potential possibilities to PSB synthesis and ensuring of the filament movement. By means of PSB

Matysik S.I., Kuzminov B.P., Ostapiv D.D. Cytotoxic action of hepatoprotector antral on bull sperm (на англ. яз.; *Матысик С.И., Кузьминов Б.П., Остапив Д.Д.* Цитотоксическое действие гепатопротектора антраля на сперму быков)
DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209
Оригинальная статья

The intensity of oxidation processes in spermatozoa and their survival under the influence of different doses of antral ($n = 4$, $M \pm m$)

Conditions of sperm incubation	Activity		Survival of spermatozoa, h
	respiratory, ng-atom O/0,1ml C · min	restorative, mV/0,1ml C · mon	
Control (PSB)	7,04 ± 0,74	0,33 ± 0,01	108,0 ± 6,00
PSB + 1/500 LD_{50}	2,90 ± 0,68*	0,11 ± 0,01*	102,0 ± 9,95
PSB + 1/100 LD_{50}	2,22 ± 0,65*	0,15 ± 0,04*	78,0 ± 5,20*
Correlation ratio, η^2	0.76	0.82	0.44

N o t e. * – probable compared to control ($0,001 > p < 0,01$).

energy, created in mitochondria as a result of the coupling of respiration and phosphorylation, cells perform specific functions, including, conduction of reaction-answer to the external influence. Although the cytotoxic influence of antral in dose 1/500 LD_{50} does not lead to changes of energy process in spermatozoa but probably does not disrupt the balance between the functional activity of the components of electron transport chain and anti-oxidant system of cells [18, 19, 22]. Excess of pharmacological drug (1/100 LD_{50}) reduces the functioning of the respiratory chain of mitochondria, violates mitochondrial oxy-reduction [20, 21], accompanied by the creation of active forms of oxygen, the result of which is a movement of spermatozoa and period of their survival on the level of the reproductive system.

Conclusions

1. Cytotoxic action of hepatoprotector antral in doses1/500 LD_{50} and 1/100 LD_{50} onoxidation processes in semen is characterized by the reduction of respiratory and restorative activity of spermatozoa.
2. Survival of the reproductive cells is preserved under the action of 1/500 LD_{50} of the drug and significantly reduces under the action of 1/100 LD_{50} of antral.
3. To substantiate the allowable level of antral content in the air of the working area, it is necessary to conduct the study of its gonadotoxic effects on laboratory animals.

References

1. Almova A.A., Begieva M.B. Definition of toxic action of polymeric materials on the basis of N,N-dasllyamino acids. *Fundamental'nyyeissledovaniya [Fundamental Research]*. 2012; 9: 539–54. (in Russian)
2. Shtolts K.F., Mosolova I.M., Dronova L.A. *Amperometric definition of ferrocyanide in the presence of subcellular structures. Biochemical methods [Antropometricheskoye opredeleniye ferrotsianida v sootvetstvii s subkletochnymi struktur. Biokhimicheskiye metody]*. Moscow: Nauka; 1980: 147–50. (in Russian)
3. Dmytrukha N.N. Cell culture as in vitro model in toxicology studies. *Medix. Anti-Aging*. 2013; 3: 50–5. (in Russian)
4. Drogovoz S.M., ShchekinaYe.G., Ushakova A.N. Modern approaches to the treatment of diseases of the hepatobiliary system. *Provizor [Pharmacist]*. 2008; (8): 19–22. (in Russian)
5. Study of the embryotoxic effect of pharmacological substances and their influence on the reproductive function. Methodical instructions. Comp. by A.P. Dyban et al. Moscow; 1989. 24 p. (in Russian)
6. Kayumov R.I., Eskov A.P., Petrova L.P. Toxicological control of perfumery and cosmetology products. Method *in vitro* on short-term suspended culture of motile cells of bull semen. *Toksikologicheski yvestnik [Toxicological Review]*. 2002; (5): 44–7. (in Russian)
7. Marchenko M.L. Culture of human cells as an alternative method in the complex toxicological and hygienic assessment of heavy metal compounds. Autoabstract of Diss. Kiev; 2011. 19 p. (in Russian)
8. Muzyka V.P., Atamayuk I.S., Chajkovska O.I., Panyx O.R., Sergiyenko O.I., Ostapiv R.D. Evaluation of toxicological effects of polymer products using test-object of bull sperm. *[Scientific and technical bulletin of the State Scientific-Research Control Institute for Veterinary Medicinal Products and Feed Additives and the Institute of Animal Biology]*.12 (3): 414–20. (in Ukrainian)
Музика В.П., Атамаюк І.С., Чайковська О.І., Паних О.Р., Сергієнко О.І., Остапів Р.Д. До оцінювання токсикологічної дії полімерних виробів з використання тест-об'єктів сперміів бугаїв. Науково-техніний бюлетень. Інституту біології тварин і ДНДКІ ветпрепаратів та кормових добавок 12 (3), 414–20.
9. Ostapiv R.D., Manko V.V., Yaremchuk I.M., Ostapiv D.D. The intensity of sperm breathing and survival of semen with adding of taurine into rarefied ejaculate of bull. *Biologiyazhivotnykh*. 2014; 16 (3): 110–5. (in Russian)
10. Plohinskiy N.A. *Biometrics: a methodical instructions for students of biological specialties. 2nd ed [Biometriya: uchebnoye posobiye dlya studentov biologicheskikh spetsial'nostey: 2-e izd]*.Moscow: Izdatel'stvo MGU; 1970. 367 p. (in Russian)
11. Skulachev V.P. Phenomena of programmed death. Mitochondria, cells and organs: the role of reactive oxygen species. *Sorosovskiy obrazovatel'nyy zhurnal*. 2001; 7 (6): 4–10.
12. Traxtenberg I.M., Kovalenko V.M., Koksharova N.V., Zhminko P.G., Chumak V.T., Baula O.P. *Alternative methods and test systems. Drug Toxicology. [Al'ternativnyye metodyi test-sistemy. Lekarstvennaya toksikologiya. Pod red. akademika AMNU I.M. Trakhtenberga]*. Moscow: Avitsenna; 2008. 272 p. (in Russian)
13. Frolov V.M., Grigoreva A.S. Antral – an effective medicine for the treatment of liver diseases. *Ukrainskiy meditsinskiy al'manakh*. 2003. 2 (34); 65–68. (in Russian)
14. Shergin N.P. *Biochemistry of spermatozoa of farm animals [Biotehnologicheskie i molekulayrno-geneticheskie osnovy vosproizvodstva zhivotnykh]*. Moskow: Kolos; 1967. 239 p. (in Russian)
15. Shtolts K.F., Mosolova I.M., Dronova L.A. *Amperometric determination of ferrocyanide in the presence of subcellular structures. Biochemical methods [Amperometricheskoye opredeleniye ferrotsianida v sootvetstvii s subkletochnymi struktur. Biokhimicheskiye metody]*. Moscow: Nauka; 1980: 147–50. (in Russian)
16. Yablonskiy V.A., Homin S.P., ZavIryuha V.I. *Biotechnological and molecular genetic bases of reproduction of animals [Biotekhnologicheskiyei molekulyarno-geneticheskiye osnovy vosproizvodstva zhivotnykh]*. Lvov: Afisha; 2009. 218 p. (in Ukrainian)
Яблонський В.А., Хомин С.П., Завірюха В.І. та ін. Біотехнологічні і молекулярно-генетичні основи відтворення тварин. Львів: Афіша: 2009. 218 с.
17. Xianglan Q., Tuyet Thi N., Seong-Kyung C. et al. Essential role of mitochondrial Ca^{2+} uniporter in the generation of mitochondrial pH gradient and metabolism-secretion coupling in insulin-releasing cells. *J Biol Chem*. 2015; 290: 4086–96.
18. Garrett L.J.A., Revell S.G., Leese H.J. ATP Production by Bovine Spermatozoa and Its Relationship to Semen Fertilizing Ability. *J Androl*. 2008; 29: 449–58.
19. Edwards S.E., Buffone M.G., Knee G.R. Effects of Extracellular Adenosine 5-Triphosphate on Human Sperm Motility. *Reprod Sci*. 2007; 14 (7): 655–66.
20. Eghbali M.A., Alavi-Shoushtari S.M., Rezaii S. Effects of copper and superoxide dismutase content of seminal plasma on buffalo semen characteristics. *Pak J Biol Sci*. 2008; 11: 1964–8.
21. Neagu V.R., Garcia B.M., Rodriguez A.M. et al. Determination of glutation peroxidase and superoxide dismutase activities in canine seminal plasma and its relation with sperm quality and lipid peroxidation post thaw. *Theriogenology*. 2011; 75: 1010–6.
22. De Lamirande E., Gagnon C. Reactive oxygen species and human spermatozoa II depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. *J Androl*. 1992; 13: 379–86.
23. Martina Doležalová, Luděk Stádník, Zuzana Biniová, Jaromír Ducháček and Jan Beran, The effect of the freezing curve type on bull spermatozoa motility after thawing, *Acta Veterinaria Brno*. 2015; 84 (4): 383–91.
24. Kosenko M.V., Chuhrij B.M., KocyumbasI.Ya. *Reproductive function and andrological dispensary of bulls [Reproduktivnaya funktsiya i andrologicheskaya dispanserizatsiya bykov]*. Lvov; 2007. 186 p. (in Ukrainian)

DOI: http://dx.doi.org/10.33029/0016-9900-2020-99-2-206-209
Original article

25. De Caimmi T., BeorleguiA., Beconi N. Oxidation de sustratos y lactatodeshidrogeasamitocondrialen semen bovinocongelado. *Rev Med Veter.* 1986; 67: 108–10.
26. Ilyuschenko V.P. Rapid testing of toxicity, based on the determination of respiratory activity of sperm and/or ciliates. *Ecology.* 1995; (1): 63–7. (in Russian)
27. Kava S.J., Yaremchuk I.M., Kuzmina N.V., Ostapiv D.D. The relationship between the physiological parameters of ejaculates and the oxidative processes in the semen of bulls. *Nauchno-tekhnicheskiybyulleten Gosudarstvennogo nauchno-issledovatel'skogo kontrol'nogo instituta veterinarnykh preparatov i kormovykh dobavok i Instituta biologii zhivotnykh [Scientific and technical bulletin of the State Scientific-Research Control Institute for Veterinary Medicinal Products and Feed Additives and the Institute of Animal Biology].* 2015; 16 (2): 367–371. (in Ukrainian)
28. Kuzminov B.P., Zazulyak T.S., Matysik S.I., Turkina V.A., Alokhina T.A. Hygienic regulation of «Antral» with justification of permissible content in the production environment of chemical-pharmaceutical enterprises. *Modern Problems of Toxicology, Food and Chemical Safety.* 2016; (4): 45–8. (in Russian)

Литература (пп. 17–23, 25 см. References)

1. Алмова А.А., Бегиева М.Б. Определение токсического действия полимерных материалов на основе N,N-диалиламиновых кислот. *Фундаментальные исследования.* 2012; 9: 539–41.
2. Штольц К.Ф., Мосолова И.М., Дронова Л.А. *Антропометрическое определение ферроцианида в присутствии субклеточных структур. Биохимические методы*. М.: Наука; 1980: 147–50.
3. Дмитруха Н.Н. Культура клеток как *in vitro* model в токсикологических исследованиях. *Medix Anti-Aging.* 2013; 3: 50–5.
4. Дроговоз С.М., Щекина Е.Г., Ушакова А.Н. Современные подходы к терапии заболеваний гепатобилиарной системы. *Провизор.* 2008; 8: 19–22.
5. Изучение эмбриотоксического действия фармакологических веществ и влияния их на репродуктивную функцию. Методические указания. Сост. А.П. Дыбан и соавт. М.; 1989. 24 с.
6. Каюмов Р.И., Еськов А.П., Петрова Л.П. Токсикологический контроль парфюмерно-косметической продукции. Метод *in vitro* на кратковременной суспензионной культуре подвижных клеток спермы быка. *Токсикологический вестник*. 2002; 5: 44–7.
7. Марченко М.Л. Культура клеток человека как альтернативный метод в комплексной токсиколого-гигиенической оценке соединений тяжёлых металлов. Автореф. дис. канд. мед. наук. Киев; 2011. 19 с.
8. Музыка В.П., Атаманюк И.Е., Чайковская А.И., Панич А.П., Сергиенко А.И., Остапив Д.Д., Яремчук И.М., Кузьмина Н.В., Остапив Р.Д. К оценке цитотоксического действия полимерных изделий с использованием тест-объєкта – спермиев быков. *Біологія тварин.* 2011; 13 (1–2): 279–285.
9. Остапив Р.Д., Манько В.В., Яремчук И.М., Остапив Д.Д. Интенсивность дыхания спермы и выживания сперматозоидов при добавлении таурина в разреженные эякуляты быка. *Биология животных.* 2014; 16 (3): 110–5.
10. Плохинский Н.А. *Биометрия: учебное пособие для студентов биологических специальностей. 2-е изд.* М.: Издательство МГУ; 1970. 367 с.
11. Скулачев В.П. Явления запрограммированной смерти. Митохондрии, клетки и органы: роль активных форм кислорода. *Соросовский образовательный журнал.* 2001; 7 (6): 4–10.
12. Трахтенберг И.М., Коваленко В.М., Кокшарёва Н.В., Жминько П.Г., Чумак В.Т., Баула О.П. *Альтернативные методы и тест-системы. Лекарственная токсикология. Под ред. академика АМНУ И.М. Трахтенберга*. М.: Авиценна; 2008. 272 с.
13. Фролов В.М., Григорьева А.С. Антраль – эффективный препарат для лечения заболеваний печени. *Украинский медицинский альманах.* 2003; 2 (34): 65–8.
14. Шергин Н.П. *Биохимия сперматозоидов сельскохозяйственных животных*. М.: Колос; 1967. 239 с.
15. Штольц К.Ф., Мосолова И.М., Дронова Л.А. *Амперометрическое определение ферроцианида в присутствии субклеточных структур. Биохимические методы*. М.: Наука; 1980: 147–50.
16. Яблонский В.А., Хомин С.П., Метелица В.И. *Биотехнологические и молекулярно-генетические основы воспроизводства животных*. Львов: Афиша; 2009. 218 с.
24. Косенко М.В., Чухрій Б.М., Коцюмбас І.Я, Репродуктивна функція і андрологічна диспансеризація бугаїв. Львів; 2007. 186 с.
26. Илющенко В.П.Быстроте тестирование токсичности, основанное на определении респираторной активности сперматозоидов и (или) инфузорий. *Экология.* 1995; 1: 63–7.
27. Кава С.Й., Яремчук І.М., Кузьміна Н.В., Остапів Д.Д. Зв'язок між фізіологічними показниками еякулятів та окисними процесами у спермі бугаїв. *Науково-технічний бюлетень Державного науково-дослідного контрольного інституту ветеринарних препаратів та кормових добавок і Інституту біології тварин*. 2015; 16 (2): 367–371.
28. Кузьминов Б.П., Зазуляк Т.С., Матысик С.И., Туркина В.А., Алёхина Т.А. Гигиеническая регламентация Антраль с обоснованием допустимого содержания в производственной среде химико-фармацевтических предприятий. *Современные проблемы токсикологии, пищевой и химической безопасности*. 2016; 4: 45–8.