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EFFECTIVENESS OF USING THE COMPLEX DRUG 'KARAFAND+OV,Zn' TO INCREASE THE REPRODUCTIVE CAPACITY OF MALES OF DOMESTIC ANIMALS

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Summary. Experimental research on male domestic animals (boars, bulls) proved the effectiveness of using the complex drug 'Karafand+OV,Zn' to increase their reproductive capacity. This preparation contains carotenoids, biologically active substances from the rhizome of marsh calamus and nanoparticles of orthovanadate gadolinium activated by europium and zinc carbonate. It was observed that the introduction of the drug increased the activity of antioxidant protection — catalase activity in the serum and erythrocytes in boars by 15.9% ($p < 0.01$) and 11.4% ($p < 0.05$), in bulls — by 7.3% ($p < 0.05$) and 12.4% ($p < 0.01$), respectively, and SOD in boars by 30.2% ($p < 0.01$), in bulls — by 16.5% ($p < 0.05$) — when reducing the intensity of lipoperoxidation processes (reducing the amount of TBA-AP in the serum and erythrocytes of males — in boars by 19.4% ($p < 0.05$) and 16.9% ($p < 0.001$), in bulls — by 25% ($p < 0.05$) and by 12.4% ($p < 0.01$), respectively). There was an improvement in sperm quality (especially in terms of motility and content of sperm with morphological abnormalities) and endocrine function of the testes — testosterone levels increased by 16.8% in boars ($p < 0.05$), and in bulls — by 44.3% ($p < 0.001$). At the same time, there was an increase in protein-vitamin-mineral metabolism in the body: the amount of vitamin A reliably increased in boars by 14.5% ($p < 0.001$), in bulls — by 7.6% ($p < 0.05$), and the content of zinc — in boars by 36.5% ($p < 0.001$), in bulls — by 16.0% ($p < 0.01$), the amount of total protein, calcium and phosphorus also increased. Thus, the obtained results allow us to recommend the complex drug 'Karafand+OV,Zn' as an effective means for increasing the reproductive capacity of males with pronounced sperm-modeling and androgen-stimulating effects and powerful antioxidant properties

Keywords: reproductive system, lipoperoxidation, nanoparticles, biologically active substances, sperm quality, hormonal background

Introduction. The main cause of reduced reproductive capacity of males is gonadopathy of alimentary deficiency and toxic genesis (Koshevoi et al., 2015, 2016; Sabeti et al., 2016; Barik, Chaturvedula and Bobby, 2019). Their occurrence is most often caused by the use of poor quality feed and water, defective diets of breeders, uncontrolled use of chemical fertilizers, etc. (Hunchak et al., 2010; Chornozub, 2013; Koshevoi et al., 2015).

The pathogenetic chain of hypofertility of males (decreased reproductive capacity) includes changes in vitamin and mineral metabolism, imbalance of the prooxidant-antioxidant system, reduced sperm quality, and negative dynamics of the hormonal background. Increasing the synthesis of active forms of biogenic elements, mainly oxygen, causes oxidative stress, which is the result of the damaging effects of the above factors (Agarwal, Makker and Sharma, 2007; Agarwal et al., 2018; Dziekońska et al., 2017; Koshevoy and Naumenko, 2020; Otasevic et al., 2020; Ritchie and Ko, 2021).

For the treatment of animals with gonadal pathology, drugs based on nanobiomaterials have been proposed, which have pronounced antioxidant and androgen-stimulating effects and increase the body's overall resistance (Karpenko et al., 2020; Koshevoy et al., 2021).

Given the etiology and prevalence of gonadopathies among breeders, the urgent scientific task is to develop ways to increase the reproductive capacity of males (Piomboni et al., 2008; Yaremchuk et al., 2017; Ribas-Maynou and Yeste, 2020).

Our work aimed to establish the effectiveness of the complex drug 'Karafand+OV,Zn' to increase the reproductive capacity of male domestic animals.

Materials and methods. The research was conducted in the laboratories of the Department of Veterinary Reproductology of the Kharkiv State Zooveterinary Academy (since 01.09.2021 — Department of Veterinary Surgery and Reproductology of the State Biotechnological University) and the Nanostructured Materials Department named after Yu. V. Malyukin of the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv).

The material of the research was males of different species of animals, in particular groups of boars ($n = 10$) and bulls ($n = 10$), which belonged to farms of different forms of ownership. Groups of animals consisted of clinically healthy males, kept on a standard diet and had free access to water, with full reproductive capacity, as established by andrological examination according to our methodology (Koshevoi et al., 2015).

The method of increasing the reproductive capacity of male farm animals included the use of a complex preparation 'Karafand+OV,Zn' containing carotenoids, biologically active substances from the rhizome of marsh calamus and nanomaterials — nanoparticles of europium-activated gadolinium orthovanadate and zinc carbonate (Koshevoi et al., 2016). Animals of the experimental groups were administered the drug at a dose of 7.5 cm³ per boar and 10 cm³ per bull, orally, once a day for 10 days.

The effectiveness of the complex drug 'Karafand+OV,Zn' was determined by changes in total protein, carotene, vitamin A, zinc, total calcium and inorganic phosphorus, the dynamics of the prooxidant-antioxidant system (content of thiobarbiturate-active products (TB-AP), glutathione (VG), catalase and superoxide dismutase (SOD) activity), testosterone levels and sperm quality.

The studied indicators were established by commonly used methods (Vlizlo, 2012). The concentration of total testosterone in the serum was determined in the V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine using the method of enzyme-linked immunosorbent assay. Statistical processing of the results was performed by Student's *t*-test (Rebrova, 2006).

Results and discussion. Analyzing previous studies and data from literature sources found a positive effect of nanobiomaterials on the body of males with gonadopathy (Koshevoi et al., 2016; Koshvoy et al., 2021; Karpenko et al., 2020; Yaremchuk et al., 2017). The results of studies on their use to improve reproducibility, indicate a significant clinical and biochemical effect of the complex drug 'Karafand+OV,Zn'.

The administration of the drug led to improved protein-vitamin-mineral metabolism — for example, the content of carotene in the serum of bulls increased by 19.8% ($2.90 \pm 0.03 \mu\text{mol/l}$, $p < 0.001$), and the amount of vitamin A reliably increased in boars by 14.5% ($p < 0.001$), in bulls — by 7.6% ($p < 0.05$), while the zinc content increased by 36.5% ($p < 0.001$) in boars, and by 16.0% ($p < 0.01$) in bulls (Fig. 1).

Mineral metabolism was characterized by an increase in the content of total calcium in the serum of boars by 6.9% ($p < 0.05$), in bulls — by 3.5% ($p < 0.05$) and inorganic phosphorus in boars and bulls by 10% ($p < 0.05$) and 8.3% ($p < 0.05$), respectively (Fig. 2). There was an increase in total protein content in boars by 2.9% ($73.8 \pm 0.62 \text{ g/l}$, $p < 0.05$), in bulls — by 5.5% ($78.7 \pm 0.48 \text{ g/l}$, $p < 0.001$).

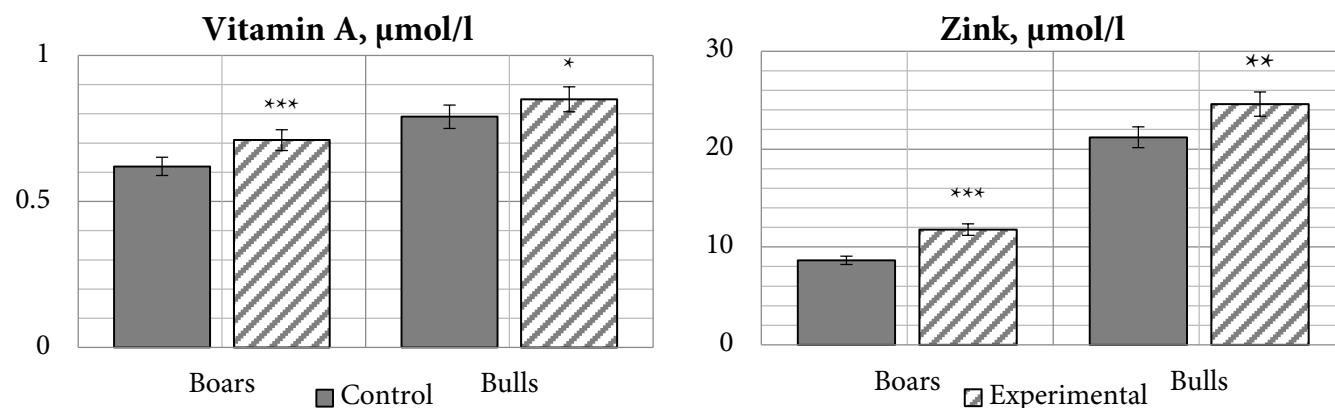


Figure 1. The effect of the complex drug 'Karafand+OV,Zn' on the content of vitamin A and zinc in the blood serum of males (* — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ compared with the control group)

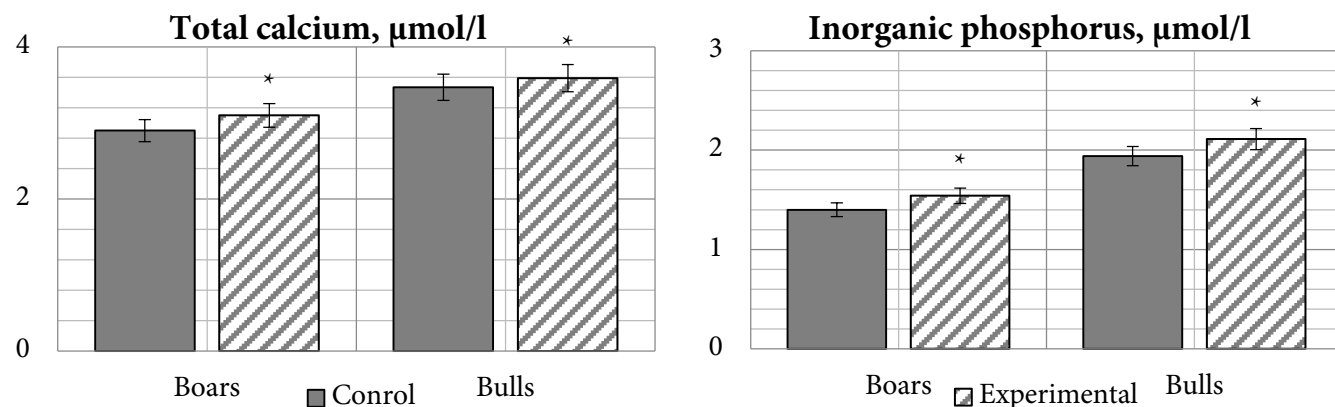


Figure 2. The effect of the complex drug 'Karafand+OV,Zn' on the content of calcium and phosphorus in the blood serum of males (* — $p < 0.05$ compared with the control group)

Activation of the antioxidant defense system and reducing the intensity of lipid peroxidation (LPO) processes have a significant impact on the body's resistance (Piomboni et al., 2008; Agarwal et al., 2018; Palani, 2018; Ritchie and Ko, 2021).

Figure 3 shows a decrease in the intensity of LPO processes after drug administration — the amount of TB-AP in erythrocytes was lower than in the control group — in boars by 16.9% ($p < 0.001$), in bulls — by 12.4% ($p < 0.01$).

The positive effect of the drug on the antioxidant status of males was noted (Fig. 4) — catalase activity in boar erythrocytes increased by 11.4% ($p < 0.05$), bulls — by 12.4% ($p < 0.01$). SOD activity increased in boars by 30.2% ($p < 0.01$) and in bulls by 16.5% ($p < 0.05$). At the same time, the content of GSH was reliably higher in boars by 3.2% ($p < 0.05$) of the control group, and in bulls tended to increase.

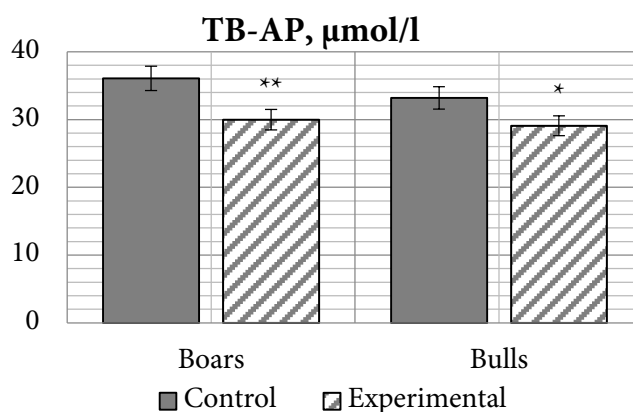


Figure 3. The effect of the complex drug 'Karafand+OV,Zn' on the intensity of lipoperoxidation in males (* — $p < 0.01$; ** — $p < 0.001$ compared with the control group)

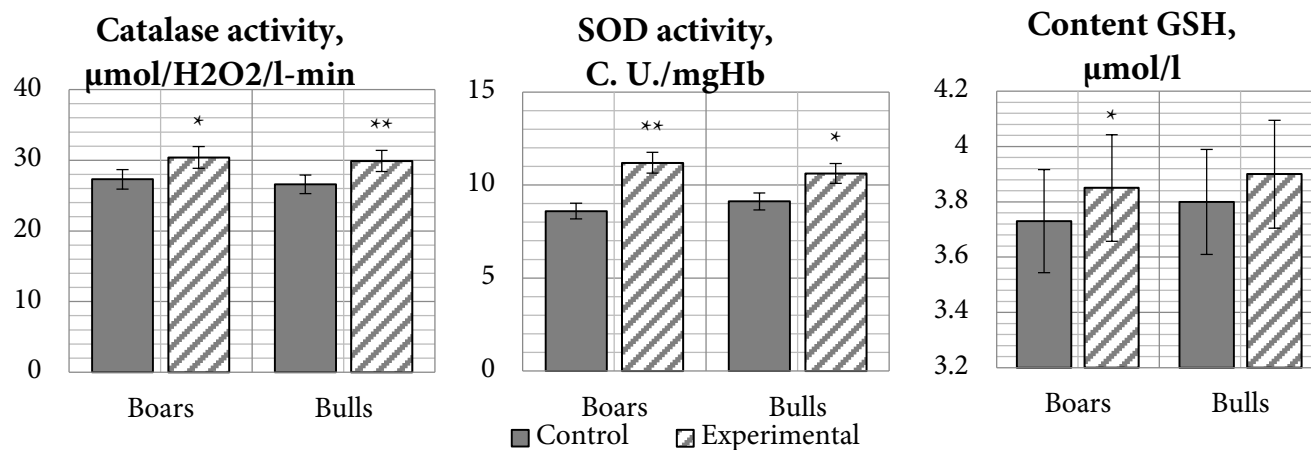


Figure 4. The effect of the complex drug 'Karafand+OV,Zn' on the antioxidant status of males (* — $p < 0.05$; ** — $p < 0.01$ compared with the control group)

Administration of the drug has a positive effect on the germinative and endocrine functions of the male gonads, which is probably due to increased resistance to oxidative stress, which is the main pathogenic mechanism of gonadopathies (Tvrdá et al., 2017; Koshevoy et al., 2021).

The method of prevention had particularly effect on the motility of sperm in ejaculate, for example, it was reliably higher in boars by 28.1% ($p < 0.05$) of the control group, and in bulls tended to increase (Fig. 5).

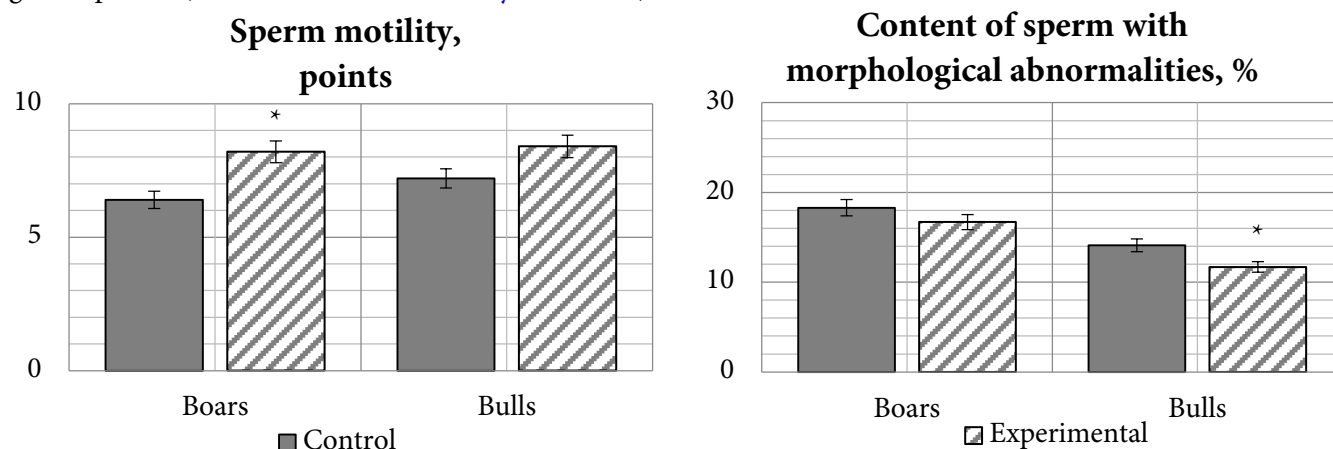


Figure 5. The effect of the complex drug 'Karafand+OV,Zn' on the quality of male sperm (* — $p < 0.05$ compared with the control group)

At the same time, there was a tendency to increase the volume of ejaculate in boars, and in bulls it reliably increased by 28.7% (4.39 ± 0.24 ml, $p < 0.05$).

The concentration of sperm in animals of the experimental groups did not change significantly, and the content of sperm with morphological anomalies was reduced by 17% in bulls ($p < 0.05$), and in boars it tended to decrease.

Testosterone levels increased by 16.8% in boars ($p < 0.05$), and in bulls — by 44.3% ($p < 0.001$), which confirms the androgen-stimulating effect of the drug (Fig. 6).

Conclusions. The obtained results allow us to recommend the complex preparation 'Karafand+OV,Zn' to prevent gonadal pathology of alimentary-deficient genesis in males. Thus, during the introduction of this drug activation of dynamics in the antioxidant defense system, improvement of protein-mineral metabolism with a decrease in the intensity of lipoperoxidation processes was observed.

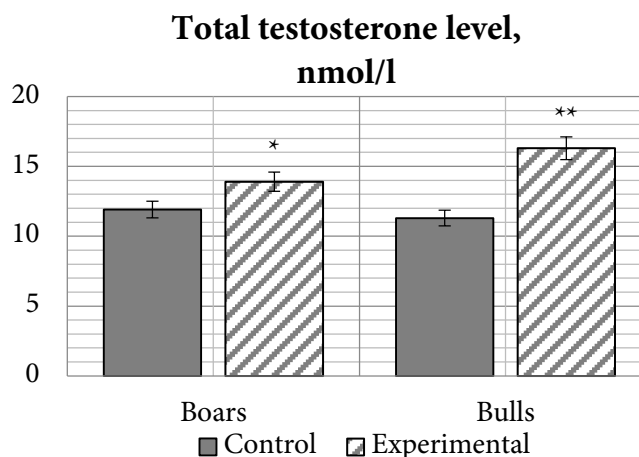


Figure 6. The effect of the complex drug 'Karafand+OV,Zn' on endocrine function of male testes (* — $p < 0.05$; ** — $p < 0.001$ compared with the control group)

References

- Agarwal, A., Makker, K. and Sharma, R. (2007) 'Clinical relevance of oxidative stress in male factor infertility: An update: Oxidative stress and male infertility', *American Journal of Reproductive Immunology*, 59(1), pp. 2–11. doi: [10.1111/j.1600-0897.2007.00559.x](https://doi.org/10.1111/j.1600-0897.2007.00559.x).
- Agarwal, A., Rana, M., Qiu, E., Al Bunni, H., Bui, A. D. and Henkel, R. (2018) 'Role of oxidative stress, infection and inflammation in male infertility', *Andrologia*, 50(11), p. e13126. doi: [10.1111/and.13126](https://doi.org/10.1111/and.13126).
- Barik, G., Chaturvedula, L. and Bobby, Z. (2019) 'Role of oxidative stress and antioxidants in male infertility: An interventional study', *Journal of Human Reproductive Sciences*, 12(3), p. 204. doi: [10.4103/jhrs.JHRS_135_18](https://doi.org/10.4103/jhrs.JHRS_135_18).
- Chornozub, T. V. (2013) *Influence of Antioxidant System Condition on Sperm Quality of Breeding Boars and Its Correction [Vplyv stanu antyoksydantnoi systemy na yakist spermy knuriv-plidnykiv ta yoho korektsiia]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Sumy: Sumy National Agrarian University. [in Ukrainian].
- Dziedońska, A., Świąder, K., Koziorowska-Gilun, M., Mietelska, K., Zasiadczyk, Ł. and Kordan, W. (2017) 'Effect of boar ejaculate fraction, extender type and time of storage on quality of spermatozoa', *Polish Journal of Veterinary Sciences*, 20(1), pp. 77–84. doi: [10.1515/pjvs-2017-0011](https://doi.org/10.1515/pjvs-2017-0011).
- Hunchak, V. M., Hufriy, D. F., Hutyy, B. V., Vasiv, R. O., Khariv, I. I., Khomik, R. I., Murska, S. D. and Guberuk, V. A. (2010) 'Influence of sodium nitrate in toxic doses on system of bull-calves' antioxidant defense and lipid peroxidation in blood of bull-calves' [Vplyv nitratu natriiu u toksychnykh dozakh na systemu antyoksydantnoho zakhystu ta perekysne oksynennia lipidiv u krovii buhatsiv], *The Animal Biology [Biolohiia tvaryn]*, 12(1), pp. 151–158. Available at: http://nbuv.gov.ua/UJRN/bitv_2010_12_1_25. [in Ukrainian].
- Karpenko, N. O., Korenieva, Ye. M., Chystiakova, E. Ye., Smolienko, N. P., Bielkina, I. O., Kustova, S. P., Boiko, M. O., Kavok, N. S., Medvedovska, N. V. and Klochkov, V. K. (2020) 'The studying of the prostateprotective and spermmodulating effects of the nanoparticles based on rare-earth elements oxides' [Doslidzhennia prostatoprotekornoj ta spermmodeliuiuchoi dii nanochastynok na osnovi oksydiv ridkiszozemelnykh elementiv], *Problems of Endocrine Pathology [Problemy endokrynoi patolohii]*, 73(3), pp. 109–118. doi: [10.21856/j-PEP.2020.3.14](https://doi.org/10.21856/j-PEP.2020.3.14). [in Ukrainian].
- Koshevoi, V. P., Naumenko, S. V., Koshevoi, V. I., Maliukin, Yu. V., Klochkov, V. K. and Kavok, N. S. (2015) 'Computer monitoring of the indicators of structural and functional conditions of the reproductive system organs in males at deficiency of carotene (vitamin A) and zinc' [Kompiuternyi monitorynh pokaznykiv strukturno-funktsionalnoho stanu orhaniv reproduktyvnoi systemy u saatsiv pry defytsi karotynu (vitaminu A) ta Tsynku], *Problems of Zooengineering and Veterinary Medicine [Problemy zooinzhenerii ta veterynarnoi medytsyny]*, 31(2), pp. 62–70. Available at: [http://nbuv.gov.ua/UJRN/pzvm_2015_31\(2\)_16](http://nbuv.gov.ua/UJRN/pzvm_2015_31(2)_16). [in Ukrainian].
- Koshevoi, V. P., Fedorenko, S. Ya., Naumenko, S. V., Ivanchenko, M. M., Onyshchenko, O. V., Besedovska, K. S., Pasternak, A. M., Hladtsinova, I. O., Koshevoi, V. I., Skliarov, P. M., Maliukin, Yu. V., Yefimova, S. L. and Klochkov, V. K. (2016) *Complex Preparations Based on Nano-Biomaterials and Their Use in Veterinary Reproductology: Methodological Recommendations [Kompleksni preparaty, stvoreni na osnovi nano-biomaterialiv, ta yikh vykorystannia u veterynarnii reproduktolohii: metodychni rekomendatsii]*. Dnipropetrovsk: Porohy. [in Ukrainian].
- Koshevoy, V. I. and Naumenko, S. V. (2020) 'The activity of the antioxidant protection enzymatic system of boars with a decrease in their reproductive capacity under oxidative stress', *Theoretical and Applied Veterinary Medicine*, 8(3), pp. 194–197. doi: [10.32819/2020.83027](https://doi.org/10.32819/2020.83027).
- Koshevoy, V. I., Naumenko, S. V., Klochkov, V. K. and Yefimova, S. L. (2021) 'The use of gadolinium orthovanadate nanoparticles for the correction of reproductive ability in boars under oxidative stress', *Ukrainian Journal of Veterinary Sciences*, 12(2), pp. 74–82. doi: [10.31548/ujvs.2021.02.008](https://doi.org/10.31548/ujvs.2021.02.008).
- Otasevic, V., Stancic, A., Korac, A., Jankovic, A. and Korac, B. (2020) 'Reactive oxygen, nitrogen, and sulfur species in human male fertility. A crossroad of cellular signaling and pathology', *BioFactors*, 46(2), pp. 206–219. doi: [10.1002/biof.1535](https://doi.org/10.1002/biof.1535).

- Palani, A. F. (2018) 'Effect of serum antioxidant levels on sperm function in infertile male', *Middle East Fertility Society Journal*, 23(1), pp. 19–22. doi: [10.1016/j.mefs.2017.07.006](https://doi.org/10.1016/j.mefs.2017.07.006).
- Piomboni, P., Gambera, L., Serafini, F., Campanella, G., Morgante, G. and De Leo, V. (2008) 'Sperm quality improvement after natural anti-oxidant treatment of asthenoteratospermic men with leukocytospermia', *Asian Journal of Andrology*, 10(2), pp. 201–206. doi: [10.1111/j.1745-7262.2008.00356.x](https://doi.org/10.1111/j.1745-7262.2008.00356.x).
- Rebrova, O. Yu. (2006) *Statistical Analysis of Medical Data: Using of STATISTICA Applied Package [Statisticheskiy analiz meditsinskikh dannykh: primeneniye paketa prikladnykh programm STATISTICA]*. 3rd ed. Moscow: MediaSfera. ISBN 5890840134. [in Russian].
- Ribas-Maynou, J. and Yeste, M. (2020) 'Oxidative stress in male infertility: causes, effects in assisted reproductive techniques, and protective support of antioxidants', *Biology*, 9(4), p. 77. doi: [10.3390/biology9040077](https://doi.org/10.3390/biology9040077).
- Ritchie, C. and Ko, E. Y. (2021) 'Oxidative stress in the pathophysiology of male infertility', *Andrologia*, 53(1), p. e13581. doi: [10.1111/and.13581](https://doi.org/10.1111/and.13581).
- Sabeti, P., Pourmasumi, S., Rahiminia, T., Akyash, F. and Talebi, A. R. (2016) 'Etiologies of sperm oxidative stress', *International Journal of Reproductive BioMedicine*, 14(4), pp. 231–240. doi: [10.29252/ijrm.14.4.231](https://doi.org/10.29252/ijrm.14.4.231).
- Tvrda, E., Mackovich, A., Greifova, H., Hashim, F. and Lukac, N. (2017) 'Antioxidant effects of lycopene on bovine sperm survival and oxidative profile following cryopreservation', *Veterinárni Medicína*, 62(8), pp. 429–436. doi: [10.17221/86/2017-VETMED](https://doi.org/10.17221/86/2017-VETMED).
- Vlizlo, V. V. (ed.) (2012) *Laboratory Methods of Research in Biology, Animal Husbandry and Veterinary Medicine [Laboratorni metody doslidzhen u biologii, tvarynnytstvi ta veterynarnii medytsyni]*. Lviv: Spolom. ISBN 9769666656776. [in Ukrainian].
- Yaremchuk, I., Kuzmina, N., Ostapiv, D., Sharan, M. and Kava, S. (2017) 'Oxidative processes intensity and quality of bull semen when adding microelements nanosuccinate compounds' [Intensyvni okysnykh protsesiv ta yakist spermiiv buhaiv za dodavannia v rozridzhuvach nanosuktsynativ mikroelementiv], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj. Series: Veterinary Sciences [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho. Serii: Veterynarni nauky]*, 19(77), pp. 185–189. doi: [10.15421/nvlvet7740](https://doi.org/10.15421/nvlvet7740). [in Ukrainian].