# MORPHOFUNCTIONAL CHANGES IN THE MALE REPRODUCTIVE SYSTEM UNDER THE INFLUENCE OF STRESSOGENIC FACTORS AND CRYOPRESERVATION

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**Abstract.** The reproductive system is permanently subjected to the action of a number of factors, which influence it positively or negatively. Therefore, the purpose of the research whose results are presented in this paper was the study of the influence of the oxidative and nociceptive stress on the semen and the reproductive system of breeder bulls and estimating the possibility of diminishing the consequences of the influence of these factors. In order to achieve the proposed purpose, microscopic, physiological, biochemical and statistical methods were used. These research methods enabled to determine that during the technological stages of cryopreservation of the bull sperm, the oxidative stress of spermatozoa causing morphological changes and decreasing the indices which characterize the functional state of reproductive cells takes place. In the case of the animals' nociceptive stress, a spermatogenesis disorder occurs, which is expressed by the tendency of spermatozoa to develop in a typical or atypical direction. Also, under the stress, the indices which characterize the functional state of spermatozoa (mobility and longevity) are decreased, and antioxidants of natural provenance can be used to reduce the negative consequences of stress. The research results have shown the action of stress at the technological stages of cryopreservation of semen and the fact that oxidative stress is one of the essential deteriorating factors in the process of sperm cryopreservation; the animals' nociceptive stress negatively influences the spermatogenesis process by conditioning an increased number of gametopathies, predetermined by reproductive cell vulnerability in the spermatogonium division phase, and lowering the functional indices of reproductive cells; the variability of cortisol content in blood plasma and malonic dialdehyde content adequately elucidates the intensity of the organism's response and cryogenic changes to the action of the stress factor; to diminish the negative consequences of oxidative stress, steroid glycosides can be included in the medium for cryopreservation as antioxidants.

**Keywords:** stress, antioxidant, reproductive cells, cryopreservation.

**Rezumat. Modificări morfofuncţionale în sistemul reproductiv masculin sub influenţa factorilor stresogeni şi crioconservare.** Sistemul reproductiv este supus permanent acţiunii unui şir de factori, care influenţează asupra lui pozitiv sau negativ. De aceea scopul cercetărilor, rezultatele cărora sunt prezentate în această lucrare, a fost studierea influenţei stresului oxidativ şi nociceptiv asupra materialului seminal şi sistemului reproductiv al taurilor reproducători şi estimarea posibilităţii de diminuare a consecinţelor influenţei acestor factori. Pentru realizarea scopului propus au fost folosite metode microscopice, fiziologice, biochimice şi statistice de cercetare, care au dat posibilitate de a stabili că pe parcursul etapelor tehnologice de conservare şi crioconservare a spermei de taur are loc stresarea oxidativă a spermatozoizilor ce provoacă modificări morfologice şi scăderea indicilor care caracterizează starea funcţională a celulelor reproductive. În cazul stresării nociceptive a animalelor are loc dereglarea spermatogenezei, care se exprimă prin tendinţa spermatozoizilor de a se dezvolta în direcţia tipică sau atipică. De asemenea, la stresare se înregistrează scăderea indicilor care caracterizează starea funcţională a spermatozoizilor (mobilitatea şi longevitatea), iar pentru diminuarea consecinţelor negative ale stresării pot fi folosiţi antioxidanţi de provenienţă naturală. Rezultatele cercetărilor efectuate au constatat acţiunea stresului la etapele tehnologice ale crioconservării materialului seminal şi că stresul oxidativ este unul dintre factorii deterioratori esenţiali în procesul crioconservării spermei; stresarea nociceptivă a animalelor influenţează negativ asupra procesului de spermatogeneză prin condiţionarea sporirii numărului de gametopatii, predeterminate de vulnerabilitatea celulelor reproductive în faza divizării spermatogoniilor, şi scăderea indicilor funcţionali ai celulelor reproductive; variabilitatea conţinutului cortizolului în plasma sanguină şi a conţinutului dialdehidei malonice elucidează adecvat intensitatea reacţiei de răspuns a organismului şi a modificărilor criogenice la acţiunea factorului stresoric; pentru diminuarea consecinţelor negative ale stresului oxidativ în calitate de antioxidanţi în componenţa mediului pentru crioconservare pot fi incluşi glicozidele steroide.

**Cuvinte cheie:** stres, antioxidant, celule reproductive, crioconservare.

## INTRODUCTION

Research by many scientists proves that a lot of factors have an extensive, negative or positive action on the male reproductive system and spermatogenesis (WANG et al., 2003; GALIMOVA et al., 2013). These factors can cause structural deviations of spermatozoa at the morphological, physiological and biochemical level. Under optimum conditions, sperm is characterized by a determined number of atypical cells; that is a normal phenomenon. An increase in this index may serve as a sign of a disorder of the functional activity of the reproductive system in different animal species, including in humans. Subjecting them to the influence of stressogenic factors can affect their organisms' homeostasis, in particular, the haematotesticular barrier and the maintenance of metabolic equilibrium in the internal testicular environment.

Physiological and cytological processes have an important role in the development of gametes. Each of these processes, at different stages of formation and development of sexual cells, may be the cause which determines their trend in the typical or atypical direction (MUNCHER et al., 1995; FURDUI et al., 2013).

One of the factors which influences spermatogenesis is oxidative stress. Oxidative stress occurs when the production of active forms of oxygen, which are potentially destructive, prevails over the level of antioxidative self-

defense. Then, this causes cell destruction. Oxidative stress is found in approximately 50% of the infertile men (BOZHEDOMOV et al., 2011). Active oxygen forms, including oxygen ions, free radicals and hydrogen peroxide, can cause infertility by two mechanisms. The first is characterized by the destruction of the membranes of spermatozoa. As a result, their mobility and ability to fertilize the egg fall. The second - by the possibility of influencing the spermatozoon DNA, which can result in the transmission of the damaged DNA information at the time of fertilization (WANG et al., 2003; BOZHEDOMOV et al., 2011). In some research, it is proven that the introduction of antioxidants can diminish the level of DNA fragmentation in spermatozoa. This confirms the hypothesis that the occurrence of DNA fractions is very often linked to oxidative stress. In addition to the above, to prevent increased DNA fragmentation in ejaculate spermatozoa, we propose using testicular sperm for fertilization (BOISSONNEAULT, 2002; WANG et al., 2003). This imbalance occurs either due to the reduction in the defence capacity of antioxidants or to the interaction with oxidative substances causing the birth of free radicals. Due to their instability, they are trying to achieve structural stability, sharing the free electron with electrophilic groups from any nearby molecule (lipids, proteins, carbohydrates or nucleic acids) (PIERCE et al., 2004). This determines a series of chain reactions that lead to the depolarization of the mitochondrial membrane, the release of cytochrome C, cause injuries to nucleic acids and oxidize the polyunsaturated fatty acids, which leads to cell death. In this process, in the regulation of apoptosis, the reactive oxygen species play an important role, being involved in both the mitochondrial, intrinsic path and the extrinsic one.

The oxidative stress caused by free radicals, such as superoxide, which easily diffuses through the cell membrane, altering the structure and functions of many cell molecules (lipids, proteins, nucleic acids), is responsible for most lesions that appear (YARIBEYGI et al., 2017).

Their consequences are multiple and include changes in mitochondrial activity, ATP destruction and apoptosis. Reactive oxygen species produce an oxidation of lipids, resulting in mitochondrial dysfunctions and negative effects on the transport of metabolites. As a result, membrane fluidity decreases, increasing their permeability to substances that cannot normally cross the membranes, and the effect is the occurrence of membrane protein lesions. Metallic cations of Cu and Fe accelerate lipid oxidation (MICLEA et al., 2010).

Free radicals and other reactive species come either from essential and normal metabolic processes or from external sources such as X-ray exposure, ozone, cigarette smoke, air pollutants, industrial chemicals, etc. A proposed classification of free radical sources is as follows: internal sources, external sources and physiological factors (GREABU, 2001).

Physiological factors refer to stress, emotions and conditions of disorders in the biological system and are responsible for producing free radicals.

A wider debate is required for a full elucidation of external sources and, in particular, internal ones. Internal sources may be enzymatic reactions that serve as free radical sources. They include the reactions involved in the respiratory chain, in phagocytosis, prostaglandin synthesis and the cytochrome P450 system. Some internal sources generating free radicals are mitochondria, xanthine oxidases, phagocytes, reactions involving iron and other transition metals, peroxisomes, the arachidonic acid metabolism pathway, physical exercise, etc. (GREABU, 2001).

External sources include non-enzymatic reactions of oxygen with organic compounds. Free radicals also appear in reactions that are initiated by ionizing radiation. Several external sources of free radicals are cigarette smoke, environmental pollutants, radiation, ultraviolet light, ozone, certain drugs, pesticides, anaesthetics and industrial solvents.

In the context of the above considerations, the proposed research purpose was to study the possibility of diminishing the negative influence of stress in the process of cryopreservation of the bull semen.

## MATERIAL AND METHODS

The research has been carried out within the Laboratory Physiology and Reproductive Health, where appropriate conditions have been organized for experiments. In the experiments, breeding bulls were included, from which during that time, with a certain periodicity, blood samples were taken and semen was harvested in all the periods: pre-experimental, experimental and post-experimental. In both groups (experimental and reference) five black-spotted bulls, aged between 1.5-1.7 years, were included. The semen, harvested before and after exposure of the males to stress, was cryopreserved according to the technological protocol. Alternating electric current with a voltage of 20 V was used as a stress factor, a method approved by the bioethics commission of the Institute of Physiology and Sanocreatology. For the determination of animal stress, the concentration of cortisol in blood plasma and the content of the final product of lipid peroxidation - malonic dialdehyde were studied.

For the determination of steroid hormones in bull blood plasma, before and after stressing animals, the specific and sensitive radiological method was used, based on the isotopic amelioration effect in the radioactively marked antiserum steroid system according to the described method (ROŞCA, 2000). Radiometry was performed by using Ultra-Beta 1280 scintillation control, produced by A/O (Sweden-Finland).

The concentration of the studied hormone was determined in standard nmol/ml units according to the following formula:$A = k * a : B * c$, where:

A – the concentration of the studied hormone in the units of measurement;

k – the content of the studied hormone in dry residues of the extract;
a – the volume of organic solvent taken for extraction;
B – the volume of the extract taken for evaporation;
c – the volume of blood serum used for extraction.

The determination of malonic dialdehyde activity in blood plasma was performed according to the procedure described by the authors (GALAKTIONOVA et al., 1998). The principle of the method consists in the following: the final product of lipid peroxidation - malonic dialdehyde (MDA) forms with thiobarbituric acid a colourful trimethyl complex, whose intensity is directly proportional to the MDA concentration in the studied sample. The optimal content of antioxidants in the synthetic medium lactose-glycerol-yolk was determined by the consecutive variability method according to (BORONCIUC & BALAN, 2008).

The determination of the content of malonic diadehyde, as one of the final products of lipid peroxidation, in reproductive cells was performed according to the Vladimirov and Arceacov method, amended for this by the Laboratory scientists with concretization of the calculation formulas (BORONCIUC & BALAN, 2008). The aim of modifying this method is to separate gametes from seminal plasma by centrifugation and to determine their concentration in the studied samples instead of protein.

As antioxidants, we used steroid glycosides obtained from different plants at the Institute of Genetics of the Academy of Sciences of Moldova and politely presented for the research.

Methods for assessing physiological indices were produced by determining in the semen the number of sperm in a unit volume (cell concentration index), the number of live sperm with rectilinear motion (mobility index) and the duration of their survival at a certain temperature (longevity index). The studied indices were determined at a temperature of 35 ºC. The estimation of pathological forms in sperm consists in determining the number of sperm with an abnormal appearance as a result of their morphological examination by the method of luminescent microscopy.

The statistical analysis of experimental data was performed by using parametric criteria by Student. The authentic differences between the reference and the experimental groups were considered to be statistically significant - $P<0.05$.

## RESULTS AND DISCUSSION

In the assisted bovine breeding techniques, cryopreserved sperm is widely used. However, freezing-defreezing makes cells much more sensitive to reactive oxygen species (ROS). When ROS are found in physiological quantities, they are involved in regulating multiple cellular mechanisms. Increased levels of ROS generate oxidative stress and lead to lesions of nucleic acids, proteins, cell membranes, and ultimately to cell death.

This phenomenon is confirmed by the data experimentally obtained at the technological stages of bull sperm freezing. The obtained results are shown in table 1.

Table 1. The contents of malonic dialdehyde in bull sperm at the cryopreservation stages.

| No. crt | Technological stage of seminal material | The final product of lipid oxidation |
|---|---|---|
| | | Malonic dialdehyde, nmol/ml |
| 1. | Dilution | 20.4±1.34 |
| 2. | Refrigeration | 30.8±3.02 |
| 3. | Freezing-Defreezing | 50.5±2.01* |

Note: * The difference is statistically significant between the technological stages of dilution, refrigeration and freezing-defreezing ($P<0.05$).

The analysis of the table data shows that at the cryopreservation of the bull sperm the content of malonic diadehyde increases 2.5 times, from 20.4 ± 1.34 to 50.5 ± 2.01 nmol / billion ($P < 0.05$), which suggests the existence of oxidative stress. At the same time, it has been demonstrated that the freezing-defreezing process reduces the glutathione concentration by 78%, and the superoxide dismutase (SOD) activity by 50% in bovine spermatozoa. Lipid peroxidation, found after sperm cryopreservation, is due to the decrease in SOD activity, characteristic of oxidative stress, that occurs during and / or after the freezing-defreezing process. The course of these processes partially explains the harmful effect of cryopreservation on the viability of the gametes. Changes in lipids due to ROS and spatial changes in membrane structures may cause various cryogenic lesions (MOREIRA et al., 2010). Sperm, in turn, generates low and controlled free radical concentrations, in particular superoxides, hydrogen peroxides and nitric oxide, as they act as mediators in ensuring capacities, hyperactivation and acrosome reaction. The latter are essential for the acquisition of the fertilization capacity of the spermatozoon and for attachment to the zona pellucida.

The continuity of research aimed at diminishing the influence of oxidative stress in the process of bull seminal material cryopreservation. For this purpose, the efficacy of steroid glycosides in the neutral synthetic medium has been studied concerning the physiological indices of defreezed gametes. The results obtained are shown in table 2.

The data presented in this table demonstrate that Petumozid-2, Rusticozid and Asparagozid-H have the clearest antioxidative property. These substances can be used in the practice of bull sperm cryopreservation.

In another series of experiments, the morpho-functional change in gametes depending on the nociceptive stress of bulls was studied. In particular, cortisol was investigated, which is a hormone produced by the body in a stressful situation, and its increased and decreased levels lead to pathologies.

Table 2. The influence of steroid glycosides on the physiological indices of defreezed bull spermatozoa.

| Name of steroid glycoside and its content, mg / 100 ml of medium | Indices of defreezed sperm | |
|---|---|---|
| | Mobility of gametes, score | Survival of gametes, hours |
| Petumozid-2 (0.125) | 3.8±0.12* / 3.4±0.10 | 5.6±0.25 / 5.2±0.58 |
| Rusticozid (0.125) | 3.8±0.12* / 3.4±0.13 | 6.8±0.20 / 6.2±0.37 |
| Melangozid (0.015) | 3.7±0.18 / 3.6±0.07 | 4.0±0.31 / 3.4±0.30 |
| Liliea-H (0.156) | 4.2±0.28 / 3.6±0.33 | 5.2±0.31 / 4.5±0.50 |
| Triozid Liliea (0.312) | 4.1±0.11 / 3.8±0.22 | 7.4±0.27* / 6.4±0.27 |
| Asparagozid-H (0.312) | 4.4±0.11* / 3.5±0.11 | 5.4±0.27 / 4.2±0.74 |

Note: * The difference is statistically significant considering comparison with the reference in the denominator, where no antioxidants were included. In brackets, the optimal glycoside content in 100 ml of medium is presented.

The concentration of cortisol determined by the adrenal and occurrring in the blood is directly involved in maintaining the nutrient balance and control of carbohydrate metabolism.

Recent data suggests the important role of cortisol in the response reactions of the organism to stress (TSIGOS et al., 2020). Due to the hormone and the reproductive system connection, in order to determine the stress state of the experimental animals, the cortisol content in the blood plasma was studied. Results of the stress conditions research are shown in table 3.

Table 3. Variations in the cortisol contents in the blood plasma of breeding bulls under stress.

| **No. crt** | **Breeding bull No.** | **Contents of cortisol, nmol/ml** | |
|---|---|---|---|
| | | Before stress (reference) | After stress (experiment) |
| 1. | I | 14.3 | 62.8 |
| 2. | II | 15.4 | 44.9 |
| 3. | III | 17.1 | 53.1 |
| 4. | M±m | 15.6±0.99 | 53.6±6.33* |

Note: * The difference is statistically significant between the experimental and the reference lot ($P<0.05$).

The results in Table 3 demonstrate the stress state of the animals because the cortisol level in the stressed bulls' blood plasma is 3.5 times higher than that in the reference lot. The concentration of cortisol in the experimental lot was 53.6±6.33 nmol/ml versus 15.6±0.99 nmol/ml in the control lot. On the basis of the increased cortisol contents in the blood plasma, which serves as an indicator of the organism's stress, it can be concluded that the experimental animals reacted to the stressful factor to which they were subjected.

Subsequent research in the post-stress period has demonstrated that there was an increase in the number of pathological cells two weeks after the stressing of the breeding bulls, especially in the first series of the experiments. A higher number of gametopathies was found over 53 days in the first series of experiments, and in the second series of experiments - over 50 days after beginning to stress the animals. The quantity of pathological gametes amounted to 86.7±2.06 and 67.4±3.01%, corresponding to both lots. The high percentage of atypical spermatozoa was preserved till the end of the experiment, which lasted 141 days. After the end of the experiments, in connection with the low quality of the semen, the bulls were sent to the slaughterhouse.

Based on the results obtained and given that the period of spermatogenesis in bulls continues about 60 days (ROȘCA, 2000; FURDUI et al., 2013), one might argue that the nociceptive animal stress causes spermatogenesis disorder at all stages of its course (the phases of division, growth, maturation). Thus, the recording of the pathological cell content threshold demonstrates the vulnerability of reproductive cells at the phase of spermatogonium division.

At the same time, the physiological status of the animal organism can also be assessed by the content of one of the products of lipid peroxidation – malonic dialdehyde in the blood plasma. The results of the spectrophotometric research of malonic dialdehyde are shown in table 4.

Table 4. The contents of malonic dialdehyde in the blood plasma of breeding bulls under nociceptive stress.

| **No.** | **Breeding bull No.** | **Contents of malonic dialdehyde (u.c.)** | |
|---|---|---|---|
| | | Before stress (reference) | After stress (experiment) |
| 1. | I | 0.340 | 0.560 |
| 2. | II | 0.360 | 0.555 |
| 3. | III | 0.340 | 0.556 |
| 4. | M±m | 0.347±0.009 | 0.557±0.006* |

Note: * The difference is statistically significant between the experimental and the reference lot ($P<0.05$).

The table data show that the content of malonic dialdehyde in the blood plasma of breeding bulls after the animals' nociceptive stress increases significantly from 0.347±0.009 to 0.557±0.006 u.c. (P<0.05). The variability of these indices confirms the stressogenic state of animals to the action of nociceptive factors.

Another research of the action of the nociceptive stress on the reproductive system is the study of the morphological status of reproductive cells after its impact. Given that in optimal conditions native sperm is characterized by a determined number of atypical cells, which is a normal phenomenon, the increase of this index may serve as a characteristic signal of a disorder of the functional activity of the animals' reproductive system. Subjecting animals to the action of nociceptive stressors leads to the loss of the ability to create the protection barrier and to maintain the balance of internal environment, including that of the reproductive system, being one of the most vulnerable.

In this context, research has been undertaken to study the influence of nociceptive stress on the occurrence of gametopathies in the seminal material of breeding bulls. The results of the study of the changes in pathological gamete occurrence in native bull sperm are shown in table 5.

Table 5. The dynamics of the amount of pathological gametes in native bull sperm after nociceptive stress.

| No. | Sperm harvesting (days) | State of gametes | |
|---|---|---|---|
| | | Normal gametes, % | Pathological gametes, % |
| 1. | The period before stress | | |
| 2. | 14 days | 86.8±1.12 | 13.2±1.12 |
| 3. | The period after stress | | |
| 4. | 7 days | 83.3±2.14 | 16.7±2.14 |
| 5. | 10 days | 69.3±2.79* | 30.7±2.79* |
| 6. | 14 days | 52.1±2.80* | 47.9±2.80* |
| 7. | 21 days | 46.6±2.97* | 53.4±2.97* |
| 8. | 28 days | 45.7±3.34* | 54.3±3.34* |
| 9. | 31 days | 44.1±3.73* | 55.9±3.73* |
| 10. | 50 days | 31.1±3.05* | 68.9±3.05* |
| 11. | 57 days | 13.3±2.01* | 86.7±2.01* |
| 12. | 71 days | 62.2±3.01* | 37.8±3.01* |

Note: * The difference is statistically significant between the experimental and the reference lot (P<0.05).

From the table data it appears that, on the 7$^{th}$ day after stress, the content of pathological gametes was 16.7±2.14% and this increase was not statistically truthful. By the 10$^{th}$ day after the animals' stress the portion of pathological spermatozoa increased to 30.7±2.79%, the difference being statistically significant if compared to that of pathological forms in the prestressoric period, when it constituted 13.2±1.12%, (P<0.05). In the later period, from the 10th day after stressing up to the 57$^{th}$ day, there was an increase in the percentage of morphologically abnormal reproductive cells, which confirms the fundamental thesis (ROȘCA, 2000), that stressogenic factors can stimulate the development of morphological spermatopathies in agricultural animals. The largest part of atypical spermatozoa was recorded on the 57$^{th}$ day after stress when it was 86.7±2.01%, (P<0.05). On the 71$^{st}$ day after stress, a significant decrease in pathological cells was found, down to 37.8±3.01%, (P<0.05). One of the most common morphological changes of sperm was recorded in the acrosomal apparatus.

During this period and in the following, the concentration of gametopathologies remained at a high level, even until the 140$^{th}$ day, after which the experiment was interrupted, and the animals, because of their high content of pathological spermatozoa, were transported to the slaughterhouse.

The research results show that in bulls subjected to nociceptive stressing, unlike the prestressoric period, the significantly increased level of pathological forms of gametes was kept on throughout the spermatogenesis process, for 2 months. After this period, their contents had been gradually decreasing, but the trend of depreciation of the seminal material was preserved for a long period, which had a negative effect on the reproductive capacity of the animals. At the same time, it has been recently reported (ROȘCA, 2000) that extreme conditions in nociceptive stress can be induced by translocation and aggregation of cytoskeletal proteins. Therefore, the recorded gametopathies can occur due to changes in polymers at the molecular level, transformations of linear and globular forms.

An inadequate reaction of the organism to extreme conditions can develop stress with serious consequences, in particular, by decreasing certain functions of the reproductive cells. The latter also predetermined the study of indices which characterize the functional state of spermatozoa under conditions of nociceptive stress. The research results are presented in table 6.

Table 6. The influence of nociceptive stress on the functional indices of bull gametes.

| No. | Experimental period | Functional indices of spermatozoa after dilution with lactose-glycerol-yolk protective medium under stress | |
|---|---|---|---|
| | | Mobility, score | Longevity at 4ºC, hours |
| 1. | Prestressoric period (control) | 9.2±0.47 | 75.4±5.17 |
| 2. | Intrastressoric period | 7.6±0.84 | 66.7±5.13 |
| 3. | Poststressoric period | 6.8±0.63* | 53.7±6.82* |

Note: * The difference is statistically significant between the prestressoric and the poststressoric period (P<0.05).

The information presented in Table 6 demonstrates that nociceptive stress in bulls causes a significant decrease in reproductive cell mobility from 9.2±0.47 points in the prestressoric period to 6.8±0.63 points in the poststressoric period, ($P<0.05$). Also, in the experimental period, the longevity of spermatozoa decreased in the range from 75.4±5.17 to 53.7±6.82 hours, respectively in the pre- and poststressoric periods.

As a result of stress, the sex glands decrease their activity (RINCÓN-CORTÉS et al., 2019). Respectively, it is natural that in connection with this there is a disorder of spermatogenesis, which in mammals is sensitive to the action of various stressors of physical and chemical nature as well as of biologically active substances. Thermal factors are some of the physical factors that influence the process of reproductive cell formation (FURDUI et al., 2013). Information on nociceptive stressing and its influence on the state of spermatogenesis in the literature was not available.

## CONCLUSIONS

1. The variability of the malonic dialdehyde content in the bull sperm freezing-defreezing process denotes the action of oxidative stress at the technological stages of cryopreservation of semen. The increased value of malonic dialdehyde indices in the animal blood confirms the stressogenic state of the organism to the action of nociceptive factors as well.

2. The nociceptive stress of bulls negatively influences spermatogenesis by conditioning a drastic increase in gametopathies, predetermined by the vulnerability of reproductive cells in the phase of spermatogonium division, and causes a significant decrease in the functional indices of reproductive cells.

3. The significant increase in the cortisol content in bull blood plasma under stress demonstrates the intensity of the organism's response to the stress factor to which the animals were subjected.

4. To reduce the negative consequences of oxidative stress, the following steroid glycosides can be included as antioxidants in the synthetic medium for cryopreservation of bull semen: Petumozid-2, Rusticozid and Asparagozid-H.

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