Comparison of morphometric and histological properties of testicles and sperm production in breeding bulls with different reaction to stress

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Abstract

The aim of this study was to determine the association between bulls with different resistance to stress and the morphological and histological characteristics of the testicles and epididymis, and quantitative and qualitative indicators of sperm production for the first three years of their use on a regional breeding holding. Based on cortisol concentrations before and after stress, bulls were divided into two groups, the first with higher stress resistance (n=9) and the second with lower stress resistance (n=7). Animals of the second group had a 11.9% higher incidence of rejected sperm due to poor quality. Animals with a higher stress resistance had a 16.6% larger relative area of the seminal canals, higher weight of the testicles, larger size of the testicles, in both length and width, higher weight of the testicular appendage, and better development of the two testicular appendages. The adaptive capacity of breeding bulls should be selectively improved to evaluate their stress resistance according to the cortisol concentrations before and one hour after stress. When completing herds, it is preferable to use animals with a higher resistance to stress.

Key words: *bulls; morphological and histological characteristics of the testicles; sperm quality*

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Introduction

In the scientific literature, there is limited information on the results of studies on stress resistance in breeding bulls for their selection to breed high-value stress-resistant animals. More attention is paid to the study of factors that cause stress in animals, particularly those that can cause the greatest damage to their health, sperm production and sperm quality (Grandin, 1997; Brito et al., 2002). An influence of high and low temperatures on reproduction function has been determined in some countries (Armstrong, 1994; de la Sota et al., 1998). The presence or absence of hypercorticism in animals under the influence of negative factors are used as test signs for determining the level of stress in the organism: transportation, thermal stress, dehorning (Jonstont and Bukland, 1976), and in these cases, the time of peak blood cortisol concentrations is determined (Jonstont and Bukland, 1976; Leining et al., 1980; Welsh and Johnson, 1981). However, such an approach does not reveal all stress mechanisms (Hahn et al., 1992). Heat stress has been found to cause sperm abnormalities, the integrity of the acrosome and plasma membrane of both fresh and thawed sperm of bulls (Ahirwar et al., 2018). In some stress-reaction studies, the activity of aminotransferases and creatine phosphate kinase was tested (Grandin, 1997; Cordoba et al., 2007). There are studies of stress reactions in the dynamics of body temperature of animals by monitoring body changes using a special sensor installed on the body to measure body temperature every 30 minutes during the day and over several days. A significant influence of thermal stress on the quantitative and qualitative signs of sperm production has been revealed, though some researchers found no significant violations of the reproduction function of animals in response to heat (Brito et al., 2002). Chrousos (2000) found that the decomposition of substances prevails over their synthesis in a young animal under stress, in order to overcome stress and restore homeostasis. Mechanisms include gluconeogenesis, processes of reamination, changes in metabolism, and blocking of somatotrophin hormone receptors. All this prevents mitosis, since the body's primary task is to save energy and obtain more energy to overcome the negative effects of stress. For these reasons, the formation of animals in early ontogenesis and their spermatogenesis can be slowed (Al-Kanaan et al., 2015). It has been revealed that under stress, the immune system is reduced and the organism can suffer from infectious diseases (Voisin, 1977).

According to some studies, cortisol levels reach a peak 10-20 minutes after the start of the stress factor action. Grandin (1997) proposed that the effects of stress on bulls and cows should be assessed if blood cortisol levels reached at least 70 ng/mL. In another study (Jonstont and Bukland, 1976), stress was caused on small Holstein bulls by transporting, dehorning and castration, and the reaction to stress was studied after 24 hours and at other times, with a cortisol peak found at 21.77±1.78 ng/mL, with a minimum of 4.1±0.17 ng/mL. It is important to establish the response of the adrenal cortex to stress and to determine how much time passes until function is normalised. In another study, the peak of cortisol and testosterone was achieved 30 minutes after the start of the stressor and remained at this level for 2–3 hours (Welsh and Johnson, 1981). However, long-term stress decreases the testosterone concentration under the depressant effect of cortisol. Other researchers (Doerr and Pirke, 1976; Welsh and Johnson, 1981) reported the inhibition of testosterone production under the influence of cortisol

during prolonged stress. Under stress, full-age bulls have a lower concentration of cortisol than young bulls. Young bulls had more sperm defects than full-age bulls, respectively 11.8 versus 8.7% (Brito et al., 2002). For the movement of sperm, the sources of energy are respiration, glycolysis and adenosine triphosphate decomposition. Adenosine triphosphate serves as short-term energy (Cordoba et al., 2007). However, during stress loads, instantaneous adenosine triphosphate consumption occurs and the organism needs effective resynthesis for enhanced activity of creatine phosphate kinase in order to restore homeostasis. This can have a negative impact on the features that characterise the quality of sperm, in particular, the physical activity of sperm. It has also been determined that under stress, the biochemical processes in sperm are inhibited, entering an anabiosis state, losing mobility, but staying alive (Brito et al., 2002).

Scientists define stress as a nonspecific, protective, neuro-humoral, adaptive reaction of the organism in response to the negative influence of environmental factors, to restore homeostasis. The behaviour of an animal in stress is the result of the combined action of the nervous and endocrine systems, which has an effect on the entire endocrine system during the stress response (Knol, 1991). Consequently, the stress response is always a reaction along the hypothalamus-pituitary-adrenal axis (Tsigos and Chrousos, 2002), and in breeding bulls even of the hypothalamus-pituitary-sperm glands axis (Malfatti et al., 2006). The hypothalamus-pituitary-adrenal axis should act acutely; otherwise, it is wrong to perceive the body's response to stress (Grandin, 1997). The study of the type of stress resistance of breeding bulls as a genetically determined feature which could be used in breeding high-value, stress-resistant bulls has not been widely studied. Only a few papers (Davis, 1993) report on the coefficient of resistance inheritance to various environmental stresses in Australian cattle (in the range 0.20–0.34), and it is noted that stress resistance still affects the accuracy of the determination of animal breeding value. Raising breeding bulls with a high resistance to stress, due to hereditary nature of the type of stress resistance, presents an opportunity to change their individual tribal value into a group sign by breeding descendants in herds. Animals with high stress resistance are able to more effectively withstand operational stresses, which can arise for various reasons in industrial technology (Jonstont and Bukland, 1976; Fisher 2001; Chernenko et al., 2017, 2018). Therefore, the solution of the stress problem in the conditions of commercial exploitation of animals remains a topical issue, since only constitutionally strong and stress-resistant animals can be healthy and high-productive, and give high-value descendants with high vitality and longevity (Katanani, 2002; Mylostyvyi and Chernenko, 2019). Since types of higher nervous activity appear in ontogenesis due to different types of stress resistance, the scientific hypothesis of this study that the individual specificities of neuro-hormonal activity in breeding bulls, in the conditions of daily operating loads, can affect their sperm production, sperm quality, morphogenesis and histological structure of the testicles and the suitability of bulls for tribal use.

Materials and Methods

The research was carried out at a Dnipro regional state-owned livestock holding, using full age Holstein breeding bulls (n=16) in the summer period. Breeding bulls were kept in individual stables with an area of 18 m^2, located under a summer canopy. The mode of reproductive load on the bulls was average - one duplet (double) contact

when the male producer gives ejaculate twice per week.

The reaction of bulls to stress was determined by studying the reactivity of the stress-factors of the hypothalamus-pituitary-adrenal axis. For this purpose, cortisol concentrations in blood were measured before stress and one hour later (Hopster et al., 1999). The choice of the method to determine bull reactions to stress was based on the fact that the bulls have tribal and monetary value, and therefore stress loads cannot be caused in the classical ways, such as injection of adrenocorticotropic hormone, adrenaline, insulin, caffeine or physical activity. In this study, the stress factor for experimental animals was planned blood sampling, as a periodical, technological measure. The stress-load was a set of factors that accompanied taking of blood: hard fixation of the animals by self-closure on the head and over the nasal ring, jugular vein squeezing, change in diet in connection with blood taking, the presence of veterinarians and other staff for taking blood, and the process of taking blood that affects animals through visual contact and the smell of blood and people, causing technological stress to animals. Blood was taken in the places where the animals were usually kept, in the morning at 6:00 am prior to feeding and drinking, when they were not affected by daily technological factors. The daily secretion rhythm of the studied hormones, which is highest in the morning, even 2–5 times higher than in the evening, was also considered (Thun et al., 1981). According to the technique, the same duration and intensity of the stressor factor was provided for each animal. Since stress at the hormonal level begins to act 10 minutes after the stress load effect (Grandin, 1997), blood was taken at the same time in all animals for 10 minutes. Blood taking was repeated in one hour, to determine the dynamics of the studied blood parameters and clinical parameters. Before the blood was taken again, the animals remained locked in the headlocks and could not eat the food they had been given and could not drink water. Experts, veterinarians and other staff remained near the animals. It was considered that the specificities of the nervous and hormonal activity of animals are found not only in achieving the maximum concentration of cortisol after stress load, but also in determining the dynamics after stress load, in comparison with initial values.

Bulls were divided into groups based on the range of reference norms (Table 1). For Holstein breeding bulls, cortisol concentrations in the morning range from 57.96–110.40 nmol/L (Kondrahin, 2004). The ratio of the maximum reference norm (110.40 nmol/L) to its minimum value (57.96 nmol/L) gives the result of 1.9, or up to 2.0. A larger value of this ratio may indicate higher reactivity of animals in response to stress. This is due to the individual characteristics of the nervous and hormonal activity and the type of the nervous system of animals (Hopster et al., 1999).

The experimental breeding bulls (CS_2/CS_1) <2.0 were distributed into the first group (n=9) that showed a higher resistance to stress. Animals with a ratio (CS_2/CS_1) ≥ 2.0 were placed in the second group (n=7) that showed a lower resistance to stress (Table 1).

The serum cortisol concentration was studied in the certified laboratory PLC Vis-Medic in Dnipro using the following equipment: microplate immuno-enzyme analyser Stat Fax-2100 (analyser adjusted to 450 nm and 600 or 620 nm wavelengths); Stat-Fax-2200 incubator shaker (adjusted for 500–700 movements per minute); automatic washbasin Stat Fax-2600; semi-automatic dispensers for 5–50, 50–200 and 200–1000 μL, as well as tips; graduated laboratory dishes; filter paper for drying strips. 96 microtiter cells (12 strips) were used; cortisol-

Table 1. Distribution of breeding bulls according to the stress resistance

Bull, Name and number	Blood cortisol concentration, nmol/L		Correlation (CS_2/CS_1)	Group based on stress resistance
	before stress load (CS_1)	after stress load (CS_2)		
Acord 4761	112.5	201.3	1.8	I
Venets 5735	73.6	100.8	1.4	I
Vorotar 3209	115.2	193.5	1.7	I
Drobovyk 2131	105.4	167.2	1.6	I
Emir 2259	120.1	163.7	1.4	I
Lenkor 8385	101.2	138.4	1.4	I
Reiner 23685	95.5	158.3	1.7	I
Khorovod 2165	58.4	105.8	1.8	I
Yug 8756	70.2	101.1	1.4	I
Adler 2175	79.3	362.6	4.6	II
Atlas 8365	89.4	550.2	6.2	II
Esaul 9747	107.5	501.3	4.7	II
Kvint 5801	109.1	253.5	2.3	II
Oval 5795	79.7	363.9	4.6	II
Sygach 2177	110.3	405.3	3.7	II
Yupiter 161	126.5	274.7	2.2	II

antiserum (blue); cortisol standards; cortisol-control; cortisol-enzyme conjugate concentrate; conjugate solvent; tetramethylbenzidine chromogenic solution; washing concentrate; stop solution and two times distilled water. The inner walls of the microtiter cells are covered with goat's anti-glare globulin serum. One 11 mL bottle of cortisol-antiserum contains rabbit anti-cortisol serum in a protein (BSA) buffer with a non-mercury preservative. One 0.5 mL bottle of cortisol-standard, labelled A, contains 0.0 µg/dL cortisol, and seven bottles labelled B-H contain cortisol in a protein buffer (BSA) with a non-mercury preservative at concentrations of 0.5, 1.5, 4.0, 10.0, 20.0, 40.0 and 60.0 µg/dL, respectively. Two 0.5 mL bottles of cortisol-control each with levels I and II contain low and high cortisol concentrations in a protein buffer with no mercury preservative. One 0.3 mL cortisol-enzyme ampule of conjugate concentrate contains a cortisol solution in a protein buffer with a non-mercury preservative. One 11 mL bottle of conjugate solvent contains a buffer with a non-mercuric concentrate based on protein. One 11 mL bottle of TMB chromogenic solution contains a solution of tetramethylbenzidine in citrate buffer with hydrogen peroxide. One 100 mL bottle with washing concentrate contains buffer salts with non-ionic detergent. One 11 mL stop bottle contains 0.2 mL sulfuric acid solution. According to the procedure, before the start of the operation, all reagents were mixed and brought to a temperature of 25°C, since

the results of optical density may be understated in the case of a lowered temperature of reagents. The principle of the determination of cortisol in serum is based on the competition between unlabelled and enzyme-labelled antigens for a certain amount of antibody bindings. At the same time, the amount of enzyme-labelled antigens bound to the antibodies is inversely proportional to the concentration of the unidentified subject. Immediately before blood taking, an enzyme conjugate solution was prepared as follows: cortisol-enzyme conjugate concentrate was diluted in a clean bowl with a conjugate solvent at a ratio of 1:50, corresponding to the number of cells. To prepare the washing solution, 100 mL of its concentrate was dissolved in 900 mL twice-distilled water. After that, it was selected to study the required number of strips with cells. To conduct a study in microplate cells, 25 μL standard, control and blood serum were introduced, then 100 μL freshly cooked enzyme conjugate solution was added. The tripod was shaken gently for 5-10 seconds to stir the content of cells. A dose of 100 μL cortisol-antiserum was introduced to each cell. For incubation at 25°C, a microtitre plate with strips was placed in an incubator-shaker for 45 minutes. Shaking at 500-700 movements was performed for 1 min. Each cell was washed five times thoroughly with an automatic washing device using 350 μL washing solution, while avoiding air bubbles. After washing, the remaining of the liquid was removed from the cells by turning the plates onto the filter paper. Using a dispenser, 100 μL TMB chromogenic solution was added to each cell. Cells were incubated in a shaker at 25°C for 10-15 minutes with a frequency of shaking of 500-700 movements for 1 min. Using a dispenser, 100 μL stop solution was added to each cell, and the dish was shaken for 5–10 seconds. After adding the stop solution, the optical density of the test solution in cells at 450 and 620 nm was measured for 30 minutes. Immediately before data was taken, the device was set to 0 in accordance with the zero standard (blank). To determine the concentration of cortisol in the samples, the values of hormone standards were used, and the standard curve constructed. Since all standards are given in μg/dL, for data conversion into nmol/L, the obtained data were multiplied by 27.6.

On average, the sperm-productive activity of breeding bulls is analysed for the first three years of their exploitation. Data from the register of sperm production and tribal registration form were used. Absolute indicators of sperm were determined by generally accepted research methods for native sperm. The following were taken into account: total volume of ejaculate; concentration of sperm in the ejaculate and total number; activity of sperm; the number of sperm dosages obtained from all ejaculates; sperm fertility ability and sperm defects.

Of the test animals, 11 of 16 were subjected to planned slaughter, some with higher (n=6) and some with lower stress resistance (n=5). The morphometric and histological characteristics of the testicles were examined. Material for research was taken after the slaughter of animals at the meat-packing plant LLC Alan of the Dnipropetrovsk region. Organometric examination of the testicles included determination of mass, volume, and dimensions: length, width and girth of each testicle. The weight of the testicles was determined by weighing on an analytical scale; the size of the testicles was determined by the volume of displaced water from the measured capacity. The mass and dimensions of the testicular appendages were determined.

To study the relationship between testicle development and the reproductive

capacity of bulls, sperm production was analysed using data from breeding record forms. The following indicators were studied: quantity of ejaculate; concentration of sperm in ejaculate; activity of sperm; number of sperm dosages obtained from all ejaculates; sperm fertilization and sperm defects.

Histological studies were carried out in the Laboratory of Histology, Immunocytochemistry and Pathomorphology of the Research Center of Biosafety and Environmental Control of Agroindustrial Resources at Dnipro State Agrarian and Economic University. Tissue pieces (1x1 cm) were cut from the testicles, fixed in 10% neutral formalin solution for 1 day, and then in 5% solution for 10 days. Samples were set in paraffin with the usual technique. Histological cuts with a thickness of 5–7 μm were made on the snuff-microtome MS-2. The slices were stained with Ehrlich Hematoxylin and eosin. Morphometric studies of histological preparations were performed according to the method of Avtandilov (1990). Using an MBS-10 microscope, the diameter of the spermatic tubules was determined, their relative area and the relative area of the interstitial tissue in 20 fields of vision at 56x magnification. Microphoto and video shooting was performed using the Olympus CX-41 microscope at 100x and 400x magnification.

To investigate the functional state of the gonads, the method to classify sperm canals with different degrees of spermatogenesis was used (Rugal, 1977): 1) tubules in which it is possible to identify all stages of spermatogenesis; 2) the total number of germ cells is not reduced, but identification is not possible; sometimes there are single degenerative cell forms; 3) the number of germ cells is sharply reduced, degenerative forms predominate among them; 4) isolated spermatogonial cells in the deep parts of the tubule, most of the cells – Sertoli; 5) only Sertoli cells with light rounded nucleus are available; 6) there are only Sertoli cells with dark hyperchromic nuclei; 7) complete (total) fibrosis.

All studies on animal were performed in accordance with the requirements of the European Convention for the Protection of Chordate Animals, used for experimental and scientific purposes (Strasbourg, 1986). The experimental data were analysed using Statistica 6.1. The data in the figures are presented as: average value, average value ± standard error, average value ± standard deviation.

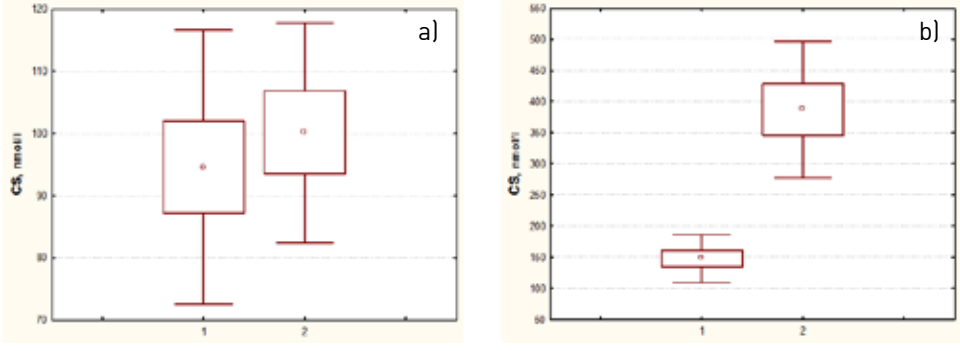

Fig. 1. Serum cortisol concentrations of cortisol before stress (a) and after stress (b) in experimental animals with different stress responses: 1 - with higher resistance to stress (*n*=9); 2 - with lower stress resistance (*n*=7); Friedman ANOVA method

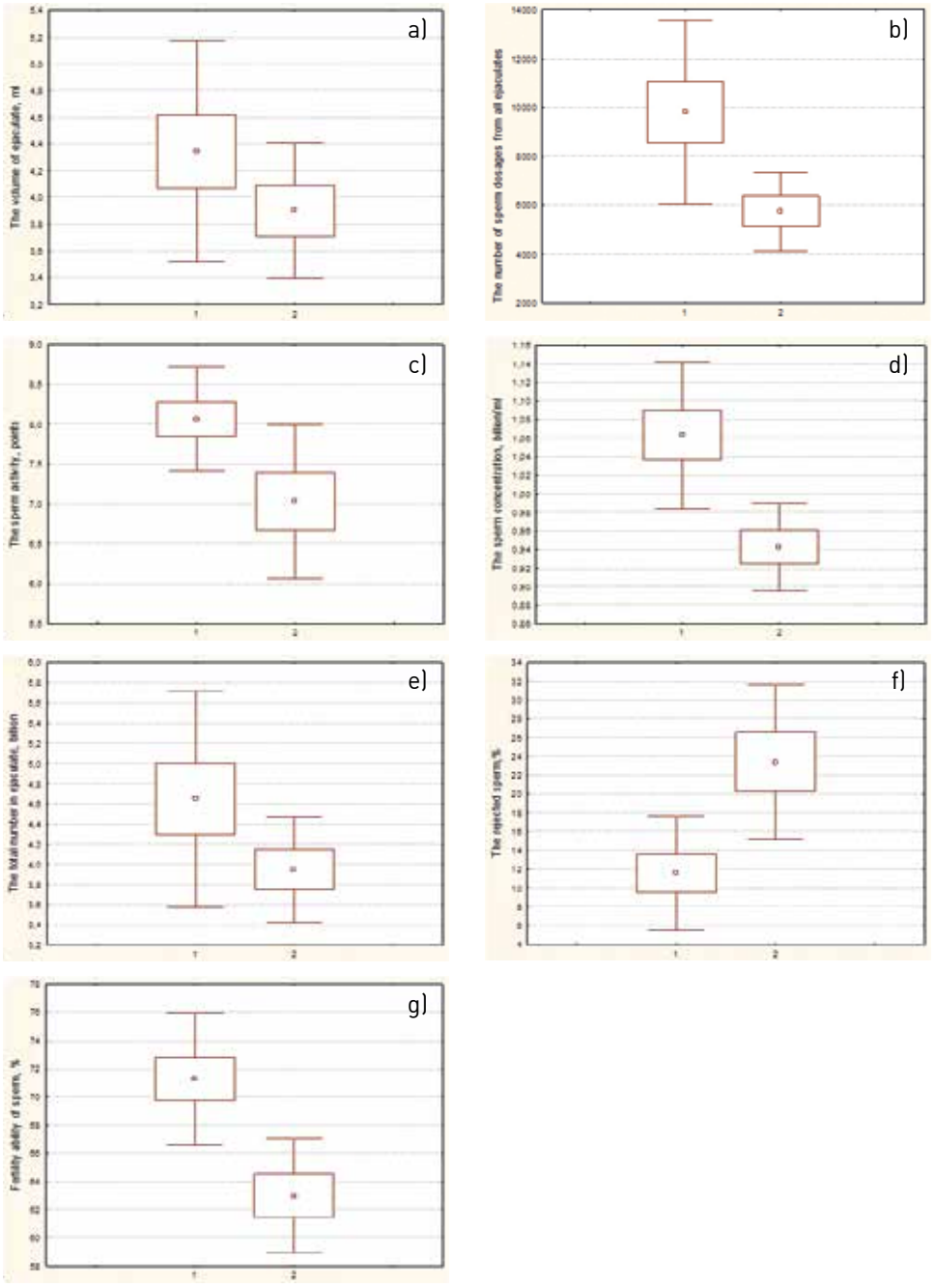

Fig. 2. Sperm production and sperm quality in experimental animals with different reactions to stress: a – the volume of ejaculate, mL; b – the number of sperm dosages from all ejaculates, c - the sperm activity, points; d - the sperm concentration, billion/mL; e - the total number in ejaculate, billion; f – the rejected sperm, %; g – fertility ability of sperm, %; 1 – with higher resistance to stress (*n*=9); 2 – with lower stress resistance (*n*=7); Friedman ANOVA method

Results

The data show that before stress, the difference between the serum cortisol concentration in breeding bulls with higher stress resistance (first group) and those with lower stress resistance (second group) was insignificant at 7.4 nmol/L (Fig. 1a). One hour after stress, an increase in serum cortisol concentrations was observed, but it was more significant in the second group. The difference was 219.5 nmol/L (P<0.001).

Breeding bulls of the first group were characterised by better indicators of sperm production (Figure 2). They had a higher concentration of sperm in the ejaculate at 0.1 billion/mL (P<0.01) (Figure 2d) and their activity was 1.0 point (P<0.05) (Figure 2c). As a result, the number of received sperm dosage from the bulls of the first group was higher by 4056.2 doses (P<0.05) (Figure 2b). The higher activity of sperm resulted in a better fertilisation capacity of sperm by

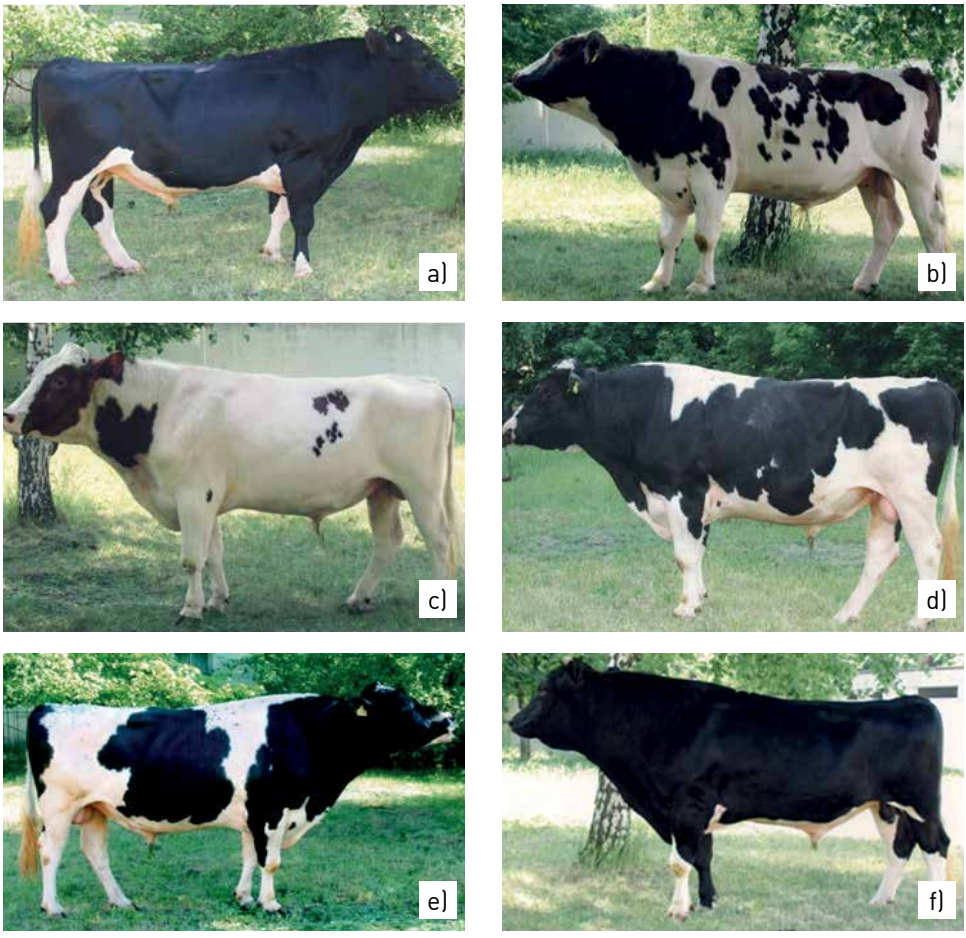

Fig. 3. Experimental breeding bulls of the Holstein breed with different reactions to stress: a - Drobovyk 2131 from Astronaut's line, the first group; b - Accord 4761 from Ayvenhou's line, the first group; c - Oval 5795 from Ayvenhou's line; the first group; d - Sigach 2177 from Elevaishn's line, the second group; e - Adler 2175 from Eleweshn's Line, second group; f - Yesaul 9747 from Chif's line, the second group

8.3% (P<0.01) (Figure 2g). Breeding bulls with a higher resistance to stress were also characterised by having a larger volume of ejaculate (Figure 2a) and the total number of sperm in the ejaculate (Figure 2e). The difference was 0.4 mL and 0.7 billion respectively. The number of rejected sperm due to lower quality was 11.9% higher in animals of the second group (P<0.01) (Figure 2f).

Figure 3 depicts the individual breeding bulls, and Figure 4 shows the histological structure of the testicles after slaughter.

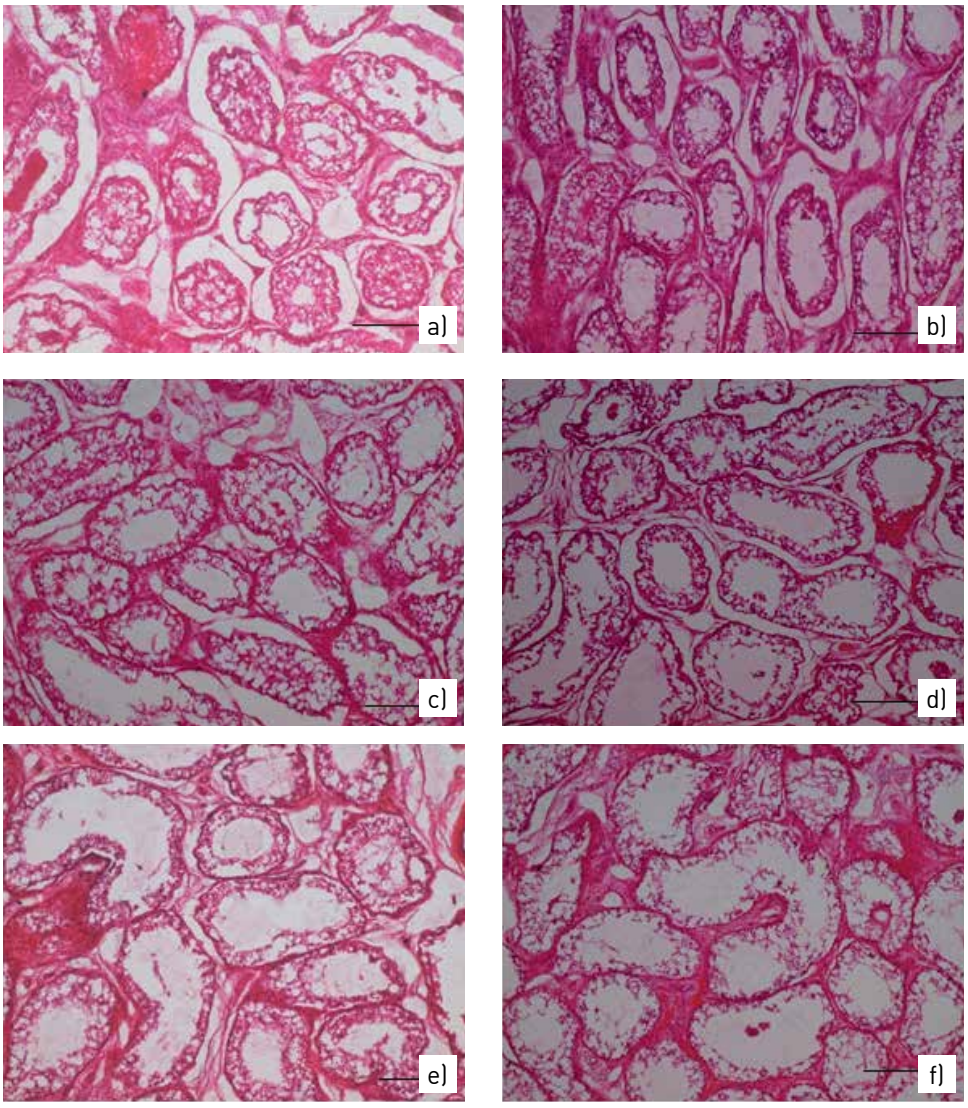

Fig. 4. Sperm tubules of experimental animals with different reactions to stress. Histological sections (stained with hematoxylin and eosin, 400x): a - Drobovyk 2131, first group; b - Accord 4761, first group; c - Oval 5795, first group; d - Sigach 2177, second group; e - Adler 2175, the second group; f - Yesaul 9747, second group; bar - 200 μm

The histological sections show the presence of twisted tubules of the correct round or round-oval form (Figure 4). The vast majority of testicles in the animals of the first group have a thickened lining, including numerous cells at different stages of differentiation (Figures 4a,b,c). Enlargement of the tubules is nearly completely filled with formed sperm. This suggests that spermatogenesis in the testicles of animals with higher resistance to stress occurred unhindered. A significant part of the sperm canals of the second group of bulls is characterized by an enlarged lumen, with a small number of sperm in the final stages of spermatogenesis, and some tubules contain cavities (Figs 4d,e,f). Some tubules showed a narrow spermatogenous epithelium, and diluted and impaired cellular elements.

As a result of the morphometric analysis of the tissues of the testicles of the breeding bulls, the differences between the groups depending on their stress resistance were determined (Figure 5).

In the breeding bulls of the first group, sperm tubules were larger in diameter by 44.6 μm (P<0.001) and the lumen diameter was smaller by 12.7 μm (P<0.05) compared with the bulls of the second group. They also have a greater relative area of the sperm tubules by 16.6% (P<0.001), and 16.6% less interstitial tissue (P<0.001) (see Fig. 5). The morphometric characteristics of the testicles and testicular appendages in experimental animals with different reactions to stress also differed (Table 2).

Breeding bulls of the first group had a greater weight of the testicles compared to those in the second group: left by 42.2 g (P<0.001) and right by 33.3 g (P<0.001), a larger volume of the testicles: left 26.2 cm^3 (P<0.001), and right by 21.1 cm^3 (P<0.05), the length of the testicles: left by 0.5 cm (P<0.05) and right by 0.5 cm (P<0.01), the girth of the testicle: left by 0.5 cm (P<0.05) and right by 0.6 cm (P<0.05), the weight of the testicular appendage: left by 9.7 g (P<0.001) and the right by 8.7 g (P<0.01), the width of both testicular appendages by 0.3 cm (P<0.001) and the length of the testicular appendage: the left by 1.3 cm (P<0.01) and the right by 1.4 cm (P<0.001) (see Table 2).

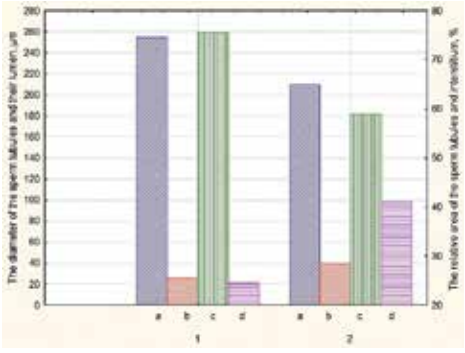

Fig. 5. The diameter of the sperm tubules (a) and their lumen (b), the relative area of the sperm tubules (c) and interstitium (d) in experimental animals with different stress responses: 1 - with higher resistance to stress (n=6); 2 - with lower stress resistance (n=5)

Discussion

From the literature (Jonstont and Bukland, 1976; Leining et al., 1980; Thomas et al., 1981; Grandin, 1997; Cordoba et al., 2007), it is known that the reaction to stress in animals is characterised by a change in blood cortisol concentration, testosterone and activity of creatine phosphate kinase and aminotransferase activity - ALT and AST. The individuality of stress resistance in animals is seen both in the maximum level of these compounds in blood after exposure to the stress factor, and in the changes to their concentrations and activity in comparison with the range of reference norms (Jonstont and Bukland, 1976; Gin et al., 2018). As a test, the determination of the level of stress in the body suggests using changes in the frequency of respiration, pulse, and body temperature (Hahn et al., 1992), however,

we were convinced that these indications did not give a clear distinction in determining the stress resistance of the bulls. Using an experimental method, we concluded that the individual specificity of the animal in the reaction to stress is clearly characterised by changes in blood parameter, especially the hormones such as cortisol.

To determine the stress resistance of breeding bulls, we decided not to include testosterone. Although cortisol blocks target cells specific to testosterone, causing aggressive behaviour and in conditions of acute stress, there is a significant increase in the blood concentration of both cortisol and testosterone, particularly at the beginning of the immobilization stage (Welsh and Johnson, 1981). However, it was defined (Knol, 1991) that the struggle for dominance, accompanied by the growth of plasma testosterone concentration as a factor of aggressiveness, was observed predominantly in the dominant bulls, while in the rest there was a decrease in this hormone. In subordinate males, this is considered an adaptation reaction, as it reduces the motivation for aggressive behaviour, to avoid conflict and stress. Thus, aggressiveness may be regarded as a reaction to stress, though as a temporary protective, adaptive reaction that is not necessarily associated with low stress resistance.

Breeding bulls with a higher response to technological stress loads may be less able to adapt to the conditions of retention, the return of sperm to the artificial vagina, the regime of sexual

Table 2. Morphometric characteristics of the testicles and testicular appendages in the breeding bulls with different responses to stress, $\bar{X} \pm SD$

Feature	Group	
	I, *n*=6	II, *n*=5
Mass of the left testicle, g	393.3 ± 10.1	351.1 ± 11.9***
Mass of the right testicle, g	401.7 ± 10.2	368.4 ± 11.9***
Volume of the left testicle, cm^3	353.3 ± 10.2	327.1 ± 10.4***
Volume of the right testicle, cm^3	354.5 ± 10.9	333.4 ± 12.9*
Width of the left testicle, cm	7.8 ± 0.1	7.7 ± 0.1
Width of the right testicle, cm	8.2 ± 0.1	8.1 ± 0.2
Length of the left testicle, cm	13.6 ± 0.3	13.1 ± 0.2*
Length of the right testicle, cm	14.1 ± 0.3	13.5 ± 0.1**
Girth of the left testicle, cm	21.9 ± 0.4	21.3 ± 0.3*
Girth of the right testicle, cm	22.1 ± 0.4	21.5 ± 0.3*
Mass of the testicular appendage of the left testicle, g	56.2 ± 2.9	46.5 ± 1.1***
Mass of the testicular appendage of the right testicle, g	57.1 ± 2.9	48.6 ± 3.8**
Width of the testicular appendage of the left testicle, cm	2.6 ± 0.1	2.3 ± 0.1***
Width of the testicular appendage of the right testicle, cm	2.7 ± 0.1	2.4 ± 0.1***
Length of the testicular appendage of the left testicle, cm	18.9 ± 0.6	17.6 ± 0.3**
Length of the testicular appendage of the right testicle, cm	19.6 ± 0.4	18.2 ± 0.3***

Note: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ANOVA method

use, and therefore, from many checked bulls, cannot create the necessary bank of sperm, and some rejected immediately after setting for testing (Fedorovych and Siraczkyj, 2007; Lockwood et al., 2017; Kasimanickam et al., 2019). An indicator of successful adaptation to environmental factors related to mechanisms of homeostasis regulation is the level of adaptive and gonadotropic hormones in the blood. However, increased secretion of adrenocorticotropic hormone during stress inhibits the synthesis of gonadotropic hormones, which leads to a decrease in the function of the reproductive system of breeding bulls (Lockwood et al., 2016). Cortisol slows the action of testosterone produced by the Leydig cells of the testicles, which may interfere with organ and tissue development, the formation of sexual dimorphism, the development of genital organs and sperm productivity of the breeding bulls, and can provoke disruptions in the sperm fertility (Luceño et al., 2019).

Reducing the stress load and its negative effects is achieved by creating conditions for the maintenance and exploitation of animals that best comply with their physiological needs (Grandin and Shivley, 2015, Mylostyvyi and Chernenko, 2019). To improve sperm quality, reduced due to the effects of technological stress, feed additives containing polyunsaturated fatty acids (PUFAs) in combination with vitamin E are given. However, if vitamin E has a beneficial effect on some sperm characteristics, then PUFA additives may have an adverse effect when applied independently. The combined application of PUFA and vitamin E did not lead to improved sperm quality (Losano et al., 2018). An assessment of the antioxidant status and oxidative stress in sperm plasma and sperm of breeding bulls of different ages in different periods of the year may have practical implications for assessing sperm quality (Vince et al., 2018). Thus, younger bulls are more susceptible to technological stress during the warm period when the higher enzyme antioxidant protection in sperm and sperm plasma was insufficient to counteract intensive oxidative processes, ultimately leading to a decrease in their mobility.

In this study, we confirmed that the different resistance of breeding bulls to stress seen in cortisol secretion affected the development of testicles and testicular appendages, histological structure, and sperm production. According to recent data (Soren et al., 2018), technological stress negatively affects testosterone secretion, since stress increases the blood cortisol concentrations (Stradaioliet el al., 2017). While preserving energy to overcome stress, this inhibits the effect of testosterone and biochemical synthesis processes in tissues. Since testosterone affects the growth and development of the gonadal gland, the disruption of its action may cause relevant morphological and histological changes in the testicles and a decrease in the processes of sperm formation and deterioration of its quality (Kastelic et al., 2018). The quality of native sperm depends on the degree of its dilution, and hence the number of sperm dosages and the activity of the sperm and their fertility (Kasimanickam et al., 2019). In addition, a violation of the synthesis of testosterone in young bulls leads to an extension of puberty (Byrne et al., 2017). According to some data (Palmer, 2016), morphological abnormalities in the testicles associated with excessive deposition of fat in the scrotum are due to the raised temperature of the testicles, thus violating their functional activity (Perumal et al., 2017). Under different conditions of keeping and use of breeding bulls, depending on the period of the year and climatic conditions, the negative effects of technological stress

on the organism of animals may differ, both in the direction of their increase and decrease, which may be the subject of the further research.

Since the dynamics of the blood cortisol concentrations of breeding bulls serve as a physiological manifestation of their adaptation to new conditions (Lockwood et al., 2017), the relevance of the study of the individual reactivity of breeding bulls to technological stresses in the conditions of the breeding establishment and the effect of this feature on their reproductive qualities become relevant. Some have stated that this is a burning issue (Fedorovych and Siraczkyj, 2007), since the premature rejection of breeding bulls on Ukrainian holdings in the past 20 years has been based on the inadequacy of their living conditions as an certain adaptation formed during a long evolution. As a result, one of the main reasons for the release of breeding bulls is unsatisfactory reproductive capacity.

In general, when completing herds with young bulls obtained from parents of high tribal value and selected for the assessment of individual qualities, animals with higher stress resistance should receive more attention at breeding holdings, in order to facilitate their better adaptation to conditions of keeping and use, good development and functional state of the sexual system and sperm production and quality of sperm.

The issue of inheritance of stress resistance level by the descendants of breeding bulls and the influence of breeding bulls with different reactions to stress on the quality of descendants is insufficiently studied. However, this is important for the formation of high genetic productivity potential in animals, and also affects the accuracy of determining their breeding value and the overall success of animal breeding, and is a prospect of further research.

Conclusions

Lower resistance of breeding bulls to stress load can disrupt morphogenesis and the functional condition of testicles and testicular appendages, and the quantitative and qualitative indices of sperm production and fertilisation capacity of sperm. Under conditions of operational load, such breeding bulls are more responsive to technological measures. This is confirmed by an increase in serum cortisol concentrations one hour after stress and in a larger CS_2/CS_1 ratio, with lower morphological and histological parameters of the testicles ($P<0.05$ ... 0.001), and poor sperm production and sperm quality ($P<0.05$ ... 0.001), compared with animals having a higher resistance to stress. The number of rejected sperm due to reasons of poor quality was 11.9% higher in the second group ($P<0.01$). Therefore, the desired bank of sperm is more likely to be created by breeding the most resistant breeding bulls.

It is important to increase the adaptive capacity of breeding bulls by means of selection. For this purpose, young bulls should be obtained from parents of high tribal value and selected for the assessment of individual qualities, and breeding bulls with high stress resistance is recommended for use in uterine herds. It is possible to evaluate their stress resistance by examining the dynamics of cortisol before stress load and one hour later on the CS_2/CS_1 ratio. Elimination of functional disorders in bulls and their consequences is possible only through improved keeping conditions, and the use of feed additives that enhance the quality of sperm.

Financial Support

This work was supported by the NDD KR Ukraine (state registration number 0120U100448).

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Rezultati morfometrijskih i histoloških studija testisa i proizvodnje sperme rasplodnih bikova s različitom reakcijom na stres

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Cilj našeg istraživanja bio je utvrditi vezu između bikova različite otpornosti na stres i morfoloških i histoloških karakteristika testisa, njihovog epididimisa, kvantitativnih i kvalitativnih indikatora stvaranja sperme za prve tri godine njihove uporabe u regionalnom rasplodnom gospodarstvu. Temeljem različite dinamike kortizola prije i nakon stresa, bikovi su podijeljeni u skupine: prva skupina većom otpornošću na stres (n=9) i druga skpina s manjom otpornošću na stres (n=7). Životinje druge skupine imale su 11,9 % veću učestalost odbačenog sjemena zbog loše kvalitete. Životinje prve skupine koje su bile otpornije na stres imale su veću relativnu površinu sjemenovoda za 16,6 %, veću masu i veličinu testisa, a i opseg testisa je bio veći. Razlikovali su se i u veličini mase testikularnih privjesaka i oba testikularna privjeska su bila bolje razvijena u žitotinja prve skupine. Predlažemo selektivno povećati kapaciteta prilagodbe rasplodnih bikova i procjenu njihovu otpornost na stres prema dinamici koncentracije kortizola prije i sat vremena nakon stresa. Prilikom remonta stada, preporučumo korištenje životinja s većom otpornošću na stres.

Ključne riječi: *bikovi, morfološke i histološke karakteristike testisa, kvaliteta sperme*