AGRICULTURAL BIOLOGY,
2019, V. 54, № 6, pp. 1196-1205
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Aspects of reproduction

УДК 636.2:591.16:612.616.2

doi: 10.15389/agrobiology.2019.6.1196eng
doi: 10.15389/agrobiology.2019.6.1196rus

QUANTITATIVE AND QUALITY INDICATORS OF SPERM PRODUCTION IN HOLSTEIN BULLS DEPENDING ON GEOMAGNETIC ACTIVITY

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Foundation for Basic Research, grant No. 18-016-00128 A
Received July 13, 2019

Abstract

A magnetic storm is one of the most important factors affecting the biological objects. The magnetic storms have profound effects at the molecular level, affecting certain cell structures. Spermatozoa are affected by various biotic and abiotic factors inside and outside the body. During the period of geomagnetic activity, the frequency of exacerbations of chronic diseases increases, the functions of a number of systems are disrupted. However, the reports on the influence of geomagnetic activity on the reproductive function, in particular on the quantity and quality of sperm, are objectively quite limited, and for agricultural species of animals such information was not found in the available literature. The objective of the work was to study the relationships between the geomagnetic activity and the sperm production quality and quantity parameters. The subject to the survey was the sperm collected from the Holstein-Friesian bull sires (n = 10, Joint-Stock Company "Head Center for the Reproduction of Farm Animals", Moscow Province, 2018). Monitoring the K-index reflecting the geomagnetic conditions was carried out according to the data from the Pushkov Institute of Terrestrial Magnetism, Ionosphere and Radio Wave Propagation RAS. The statistical analysis was performed with SPSS v.15.0 for a one-way analysis of variance. The variables between the groups for $K \leq 1.0$ (no geomagnetic disturbance) and $K \geq 5.0$ (geomagnetic storm) were compared with the Scheffe's test. The assessment of the sperm quality was performed with the Argus-CASA software (ArgusSoft, Russia) with Nikon Eclipse Ni microscope (Nikon, Japan). Our data indicate that during the survey, the K-index was a factor affecting the biological adequacy of the bull sires. The results prove that the magnetic storm has a significant impact on the qualitative and quantitative parameters of the bull sire sperm production. We have established the statistically valid criteria for the ejaculate volumes ($F = 6.49$; $p < 0.05$) and the progressively motile sperm number ($F = 8.36$; $p < 0.05$) per ejaculate. The volume of the ejaculates of the bull sires tends to a 28.2 % decrease ($p < 0.001$) during the magnetic storm period (K-index value ≥ 5.0 vs. K-index ≤ 1.0). The geomagnetic activity causes a 11.3 % decline ($p < 0.001$) in the sperm activity.

Keywords: magnetic storm, spermatozoa, sperm motility, morphology, chromatin, nDNA, DNA fragmentation index, bull sires, CASA

Magnetic storms are one of the natural abiotic factors affecting living organisms [1, 2]. The details of the dependence of biological processes on the state of the geomagnetic field have not been fully studied yet but it is recognized that most physiological rhythms are synchronized with solar and geomagnetic activity [3-6]. At the same time, both in individual cells and in the body as a whole, the reaction to external influences depends not only on their nature and strength but also on the properties of the biological objects themselves [7].

During magnetic storms, chronic diseases of the cardiovascular and central nervous systems become more acute, blood flow is disrupted [8-10], the adrenal secretion of adrenaline increases markedly, and the state of the autonomic nervous system changes, which regulates the work of internal organs and glands of internal secretion [11]. The change in geomagnetic activity is associated with the production of melatonin [12, 13], which is involved in the regulation of circadian rhythms, improves the work of the endocrine [14], immune [15], and reproductive [16, 17] systems.

The influence of geomagnetic activity at the cellular level is explained by changes in the state and functions of cell membranes, disruption of transmembrane transport, the formation of free radical lipid oxidation products, and a decrease in the buffer capacity of the antioxidant system [18]. At the same time, data on magnetic field effects on cells are contradictory. Thus, the static magnetic field did not cause oxidative stress in mouse fibroblasts and even led to some increase in antioxidant activity [19]. Some cell compartments, such as mitochondria and endoplasmic reticulum, are more sensitive to magnetic fields [20].

Spermatozoa are affected by various biotic and abiotic factors inside and outside the body [21-23]. Most of these factors have an indirect and direct impact on the reproductive qualities of bull sires. The influence degree of external factors on spermatozoa depends on the nature of these factors and the state of the spermatozoa themselves. External factors may cause changes in morphology, nucleus, acrosome, mitochondria, and other structures. Electromagnetic fields have a significant influence on sperm motility and morphology [24, 25].

The most important indicator that characterizes the biological full-value of spermatozoa is the state of nuclear DNA in chromatin, on which male fertility depends largely. Numerous biotic and abiotic factors influence the degree of sperm nuclear DNA fragmentation [26].

Thus, numerous, mostly medical, observations indicate the dependence of the physiological state, functional activity and risks of exacerbation of some pathologies on the impact of magnetic storms on the body, but reports on the impact of geomagnetic activity on reproductive function, in particular on the quantity and quality of sperm, are objectively quite limited. We have not found such information in the scientific literature for agricultural species of animals, although in modern livestock breeding, based on the large-scale use of cryopreserved sperm and artificial insemination, the problem of a sufficient number of biologically full-fledged sperm of sires is very acute. An in-depth study of this topic in agricultural species is also important for understanding the effect of magnetic storms on male germ cells in vivo (on the body level), in addition to identifying the mechanisms of cellular responses to magnetic and electromagnetic fields in vitro.

In this paper, we show that a magnetic storm has a statistically significant effect on the volume of ejaculate ($F = 6.49$, $p < 0.05$) and the progressively motile sperm number ($F = 8.36$, $p < 0.05$) in Holstein bulls. During a magnetic storm, the volume of ejaculate decreases by 28.2% ($p < 0.001$), and sperm activity decreases by 11.3% ($p < 0.001$).

The purpose of the study was to determine whether the quantitative and qualitative indicators of sperm production in cattle change under the influence of geomagnetic activity.

Techniques. A group of 10 Holstein bulls (JSC Head Center for the Reproduction of Farm Animals, Moscow Region, 2018) aged 3-5 years with a live weight of 900-1100 kg was tested. The conditions for feeding and keeping animals were similar and corresponded to the developed standards (Ernst Federal Science Center for Animal Husbandry). Sperm collection was carried out by the operators of JSC Head Center for the Reproduction of Farm Animals following the national technology of freezing and using the sperm of bull sires [27]. Two ejaculates were taken from each bull with an interval of 10-15 minutes. Depending on the concentration, the ejaculates were diluted with OPTIXcell™ synthetic medium (IMV Technologies, France). The volume of ejaculates was taken into account and quality indicators of sperm were determined on freshly collected samples. Three samples of each ejaculate were examined (the total sample size was 360 samples).

To determine the concentration and motility of spermatozoa, the authors used Makler's chamber (sperm counting chamber) (Sefi Medical Instruments, Israel) and a computerized analysis system, a Nikon Eclipse Ni microscope equipped with a Nikon DS-Qi2 camera with high resolution (4908×3264) (Nikon, Japan) (Argus-CASA — Computerized Assisted Semen Analysis software, ArgusSoft, Russia). For motility analysis, data were obtained for at least 300 spermatozoa. The following indicators were evaluated: VAP (average path velocity, mm/s) is the average velocity of head movement average path, ґm/s; VSL (straight-line velocity, ґm/s) is the speed of rectilinear movement of the head, μm/s (average velocity of a sperm head movement along the straight line segment between the start and end point of the trajectory); VCL (curvilinear velocity, μm/s) is the actual speed of the spermatozoa movement in a curvilinear path, μm/s, ALH (amplitude of lateral head displacement, ґm) is the average deviation of the head, μm (amplitude of the lateral displacement of the spermatozoa head relative to the path of motion); BCF (beat-cross frequency, Hz), the averaged vibrational movements frequency, Hz (average frequency of intersection of the curved trajectory of the sperm cell with its average trajectory per unit of time); STR (straightness, VSL/VAP) is a measure of the straightness of spermatozoa directional movement (average of the trajectory, %; LIN (linearity, VSL/VCL, %), the degree of the tracks undulation (oscillations of the true motion path to the average trajectory), %.

When preparing smears for morphological studies, a drop of sperm was applied to a defatted glass slide, distributed with a plastic spatula in a thin layer and dried at room temperature, and the resulting preparations were stained (Quick-Diff kit, Abris+, St. Petersburg) following the attached protocol. The preparations were analyzed automatically using the Argus-CASA software following strict Krueger's criteria. The results were expressed in absolute values (the number of spermatozoa with abnormal morphology), as well as a percentage (the ratio of spermatozoa with abnormal morphology to the total number of spermatozoa, expressed as a percentage).

DNA fragmentation in spermatozoa (Sperm Chromatin Dispersion Test, SCD-test) was studied using the Halosperm® kit (Laboratories INDAS S.A.U., Spain) according to the attached instructions. Microscopy was performed at a magnification of ×40 using an Altami LUM-2 microscope equipped with a UCMOS14000KPA digital camera (Russia).

Depending on the halo size, spermatozoa were divided into five classes: without DNA fragmentation for large halo, medium halo; with fragmented DNA for small halo, no halo; degenerate for the nucleus of an unusual shape or poorly colored. Classification according to the degree of fragmentation was performed using the Argus-CASA software. The percentage of spermatozoa with different degrees of DNA fragmentation was calculated automatically.

Statistical analysis was performed with IBM SPSS Statistics 15.0 software (IBM Corp., USA). The mean values (*M*) and standard errors of means (±SEM) were calculated. To assess the significance of the influence of the geomagnetic activity factor on spermatozoa parameters, a single-factor dispersion analysis (ANOVA) was performed, using F-test and determining p-significance. To identify the difference between groups, the Scheffe method of multiple comparisons was used. The differences were deemed statistically highly reliable at $p < 0.001$, , at $p < 0.01$ and $p < 0.05$ − reliable.

Results. For 8 months of observation (from January to October 2018), according to the Pushkov Institute of Terrestrial Magnetism, Ionosphere and Radio Wave Propagation RAS (Moscow) (http://geodata.izmiran.ru/), the magnetic storms with a K-index ≥ 5.0 (the K-index of the geomagnetic situation characterizes the deviation of the Earth's magnetic field from the norm during a 3-hour interval; it has values from 0 to 9) were recorded in the study area 4 times (February 27, April 20, June 25, and August 27).

Table 1 shows the average seminogram data of the studied bull sires in the dynamics depending on the geomagnetic activity.

1. Average seminogram values of Holstein bulls depending on geomagnetic activity K-index (*M*±SEM, JSC Head Center for the Reproduction of Farm Animals, Moscow Province, January-October, 2018)

Bull No.	Volume, ml	PR, %	NP, %	IM, %	VAP, μm/s	VSL, μm/s	VCL, μm/s	ALH, μm	BCF, Hz	STR, %	LIN, %
1	5.1±0.12	88.3±2.6	7.2±0.9	4.5±0.3	89.5±1.9	77.0±1.5	127.5±3.7	5.3±0.9	28.5±1.1	80.0±3.2	62.5±0.9
	4.2±0.11	77.5±1.9	17.5±1.1	5.0±0.1	78.5±2.4	66.5±2.3	110.5±2.9	4.5±0.8	22.0±0.8	70.5±1.9	61.5±1.7
2	4.8±0.11	82.3±2.1	5.3±0.6	12.4±0.2	80.3±1.5	65.3±1.9	111.3±2.7	4.5±0.3	26.3±1.3	75.3±2.7	58.7±0.9
	3.8±0.08	71.3±1.8	15.6±1.2	13.1±0.2	69.5±1.8	59.7±3.5	100.4±3.5	3.9±0.7	20.5±0.8	72.5±1.5	60.3±1.1
3	5.1±0.12	86.2±2.2	6.8±1.1	7.0±0.1	88.2±2.7	72.4±1.8	125.5±4.7	5.2±0.9	28.1±1.1	80.0±2.1	61.8±0.5
	4.0±0.08	73.2±1.9	19.5±1.6	7.3±0.2	74.2±1.6	60.4±3.4	105.0±1.9	4.4±0.8	22.0±0.9	74.5±3.4	57.5±1.7
4	5.2±0.09	84.1±1.9	6.7±0.9	9.2±0.3	81.2±1.9	69.0±1.9	109.4±2.4	4.7±0.2	26.8±0.7	76.0±1.9	63.5±2.5
	4.2±0.16	70.3±1.9	19.9±1.8	9.8±0.1	74.3±2.7	59.5±2.5	104.0±3.5	4.2±0.3	22.6±1.1	68.5±0.8	58.1±1.9
5	4.2±0.11	82.3±2.3	5.3±0.9	12.4±0.2	81.8±1.6	73.5±1.5	120.0±2.5	5.1±0.3	29.2±0.9	78.0±1.5	62.8±0.9
	3.4±0.12	72.2±1.9	14.6±1.1	13.2±0.2	73.5±2.5	64.7±3.7	107.8±2.6	4.5±0.1	21.3±1.2	74.2±2.4	61.0±2.5
6	4.3±0.13	85.2±1.4	6.4±0.9	8.4±0.1	86.6±1.9	75.6±1.9	119.7±1.8	5.0±0.2	27.5±0.8	81.0±2.8	63.8±1.9
	3.4±0.11	74.2±1.9	16.8±1.5	9.0±0.1	72.2±1.7	62.0±2.8	109.5±2.9	4.3±0.2	20.5±0.9	73.1±3.6	57.2±2.5
7	4.9±0.12	88.2±2.6	7.8±1.1	4.0±0.2	88.3±2.4	75.0±3.6	123.0±3.8	5.2±0.1	28.0±1.6	80.0±1.9	62.5±1.5
	4.0±0.11	77.5±1.9	18.0±0.8	4.5±0.1	77.3±1.6	60.4±1.9	104.5±1.5	4.4±0.2	21.1±0.9	73.0±0.8	58.1±2.7
8	5.2±0.12	87.2±2.3	6.9±0.6	5.9±0.2	88.5±1.7	70.3±2.5	117.0±2.3	4.6±0.2	26.4±1.5	74.0±1.1	60.1±0.8
	4.1±0.11	76.5±2.1	17.0±1.1	6.5±0.1	77.5±3.5	64.5±1.9	108.4±4.9	4.1±0.1	21.0±1.7	73.2±2.7	60.1±0.9
9	4.6±0.12	87.5±3.6	7.6±0.9	4.9±0.2	88.0±2.8	70.0±1.8	116.0±3.8	4.8±0.2	26.5±0.8	75.0±0.9	60.4±1.8
	3.2±0.15	76.2±1.9	18.4±1.4	5.4±0.1	77.5±1.6	65.0±3.1	107.0±1.3	3.9±0.2	20.6±1.1	72.0±2.6	60.3±2.7
10	4.8±0.16	85.1±2.5	7.2±0.9	7.7±0.1	85.0±1.9	67.6±2.5	115.0±2.5	4.4±0.2	26.1±2.1	76.5±1.5	58.8±0.9
	3.3±0.08	74.5±1.8	17.5±1.8	8.0±0.2	73.0±3.8	62.0±3.4	104.0±3.5	3.8±0.2	20.4±0.9	72.3±2.4	58.1±1.7

Note. PR — progressive motile; NP — non-progressive motile; IM — stationary; VAP — the average speed of head movement along the average trajectory, VSL — the speed of rectilinear movement of the head, VCL — actual speed of sperm movement along the real trajectory, ALH — average deviation of the head, BCF (beat-cross frequency) — frequency of oscillatory averaged movements, STR — degree of straightness of the directed movement of sperm, LIN — degree of undulation tracks. Above the line indicators at K ≤ 1.0, below the line at K ≥ 5.0.

The ANOVA analysis showed that the magnetic storm had a reliable significant effect on the volume of ejaculate ($F = 6.49$, $p < 0.05$) and the progressively motile sperm number ($F = 8.36$, $p < 0.05$) in the ejaculate of bull sires (Table 2). During the magnetic storm, the volume of ejaculate received decreased by 28.2% ($p < 0.001$, the Scheffe test, see Table 2) compared to that obtained during the period when the magnetic situation was normal and the K-index ≤ 1.0. Under the influence of a magnetic storm, when the geomagnetic activity index was 5 points or higher, sperm motility decreased by 11.3% ($p < 0.001$). Increased geomagnetic activity is accompanied by an increase in the proportion of spermatozoa with affected and oscillatory movement. On days with disturbed geomagnetic conditions, the parameters characterizing the activity of spermatozoa reduced significantly: for VAP by 10.9 μm/s ($p < 0.01$), for VSL by 9.1 μm/s (p < 0.01), and for ALH by 0.7 μm/s (p < 0.01) (see Table 1).

2. The volume of ejaculates and sperm motility in ejaculates of Holstein bulls during periods of different geomagnetic activity (*M*±SEM, *n* = 360, JSC Head Center for the Reproduction of Farm Animals, Moscow Province, 2018)

Indicator	Geomagnetic activity index	
	K≤ 1.0 (quiet geomagnetic environment)	K≥ 5.0 (magnetic storm)
Volume, ml	4.82±0.14	3.76±0.15***
PR, %	85.60±1.20	74.30±1.50***
NP, %	6.70±1.30	17.50±1.90**
IM, %	7.70±1.80	8.20±2.10
VAP, μm/s	85.70±1.60	74.80±2.10**
VSL, μm/s	71.50±2.30	62.40±1.70**
VCL, μm/s	118.40±3.20	106.10±2.10**
ALH, μm	4.90±0.10	4.20±0.20**
BCF, Hz	27.30±0.90	21.20±1.20**
STR, %	77.70±3.10	72.40±2.40**
LIN, %	61.50±2.50	59.20±1.70*

Note. PR — progressive motile; NP — non-progressive motile; IM — stationary; VAP — the average speed of head movement along the average trajectory, VSL — the speed of rectilinear movement of the head, VCL — actual speed of sperm movement along the real trajectory, ALH — average deviation of the head, BCF (beat-cross frequency) — frequency of oscillatory averaged movements, STR — degree of straightness of the directed movement of sperm, LIN — degree of undulation tracks. Above the line indicators at K ≤ 1.0, below the line at K ≥ 5.0.
*, **, *** Differences with indicators at K ≤ 1.0 are statistically significant at p < 0.05, p < 0.01 and p < 0.001, respectively.

3. Single-factor variance analysis of frequency (%) of sperm morphology abnormalities in ejaculates of Holstein bulls as per K-index groups in periods of different geomagnetic activity (*M*±SEM, *n* = 360, JSC Head Center for the Reproduction of Farm Animals, Moscow Province, 2018)

Pathology type	Comparison	SS	df	MS	F	p
Spermatozoa pathological forms	Between groups	84.535	2	42.267	3.509	0.039
	Within groups	493.897	34	12.046		
	Total	578.432	36			
Head pathology	Between groups	0.200	2	0.100	0.385	0.699
	Within groups	1.300	34	0.260		
	Total	1.500	36			
Neck pathology	Between groups	26.449	2	13.224	1.515	0.234
	Within groups	296.794	34	8.729		
	Total	323.243	36			
Tail pathology	Between groups	6.451	2	3.225	2.324	0.034
	Within groups	19.432	34	1.388		
	Total	25.882	36			

Note. SS — the sum of squares, df — number of degrees of freedom, MS — mean square, F — Fisher test, p — statistical significance; Group I for K ≤ 1, Group II for K ≥ 5.

One-factor analysis of variance of morphological changes in spermatozoa depending on geomagnetic activity showed that the differences between the groups by average values for the content of all pathological forms and pathologies of the spermatozoa filament were reliable, and the levels of statistical significance were p = 0.039 and p = 0.034, respectively (Table 3). The statistical significance of the F-criterion for the incidence of the pathology of a head p = 0.699 and a midpiece p = 0.234 > 0.05; therefore, for these indicators, the zero hypothesis of the difference between the groups is not refuted.

On days with increased geomagnetic activity, the number of spermatozoa with pathology increased. At K ≥ 5.0 (magnetic storms), their share was 8.14%, which is 59.9% more than at K ≤ 1.0 (calm geomagnetic situation). Statistically significant differences in the frequency of filament pathologies were revealed (Fig. 1) depending on the geomagnetic activity. During a magnetic storm, the number of such sperms increased by 40.7% (p < 0.05), which led to an increase in the number of cells with affected oscillatory movement (non-progressive motile cells) (Table 4).

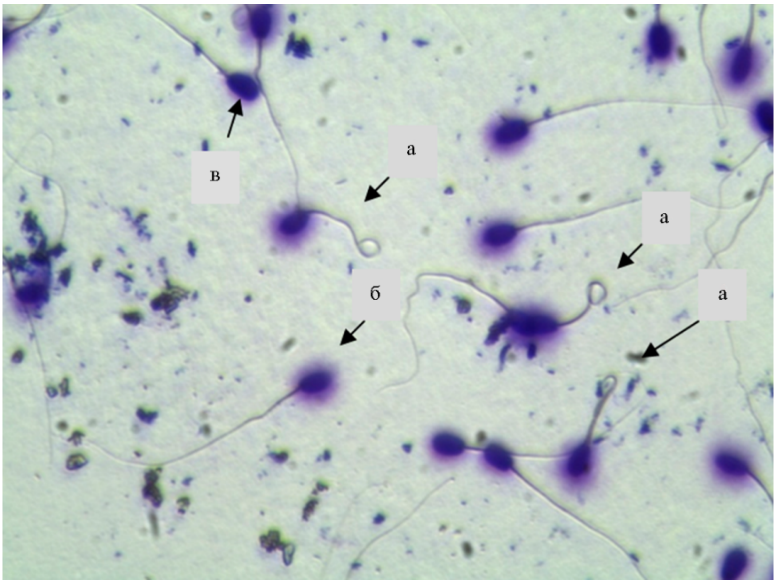

Fig. 1. Sperm Chromatin Dispersion Test (SCD-test) **in Holstein bulls:** a — tail pathology, b — spermatozoid without DNA fragmentation, c — spermatozoid with fragmented DNA (JSC Head Center for the Reproduction of Farm Animals, Moscow Province, 2018). Microscope Altami LUM-2 with a digital camera UCMOS14000KPA (Russia), ×40 zoom).

4. The proportion of sperm abnormalities in ejaculates of Holstein bulls as per K-index groups in periods of different geomagnetic activity (M±SEM, n =360, JSC Head Center for the Reproduction of Farm Animals, Moscow Province, 2018)

Indicator, %	Geomagnetic activity index	
	K ≤ 1.0	K ≥ 5.0
Spermatozoa pathological forms	4.88±0.55	8.14±0.36*
Head pathology	1.25±0.16	1.28±0.19
Neck pathology	1.64±0.30	2.30±0.24
Tail pathology	4.51±0.49	6.35±0.35*

* Differences with indicators at K ≤ 1.0 are statistically significant at $p < 0.01$.

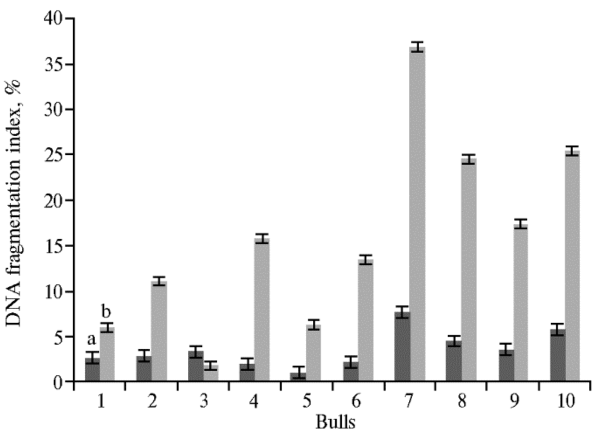

Fig. 2. Sperm DNA fragmentation index in ejaculates of Holstein bulls in periods of different geomagnetic activity: a — at K ≤ 1.0; b — at K ≥ 5.0 (JSC Head Center for the Reproduction of Farm Animals, Moscow Province, 2018).

In samples obtained from bull sires on days when magnetic storms were recorded, the fragmentation index of nuclear DNA exceeded the values at K ≤ 1.0 significantly (Fig. 2).

Studies of the impact of the magnetic storm on biological objects indicate the existence of a close dependence of physiological rhythms on geomagnetic activity [4-6]. Our investigation of the connection between the biological integrity of sperm in ejaculates and geomagnetic activity shows that magnetic storms negatively affect the quantitative (decrease in the volume of ejaculate by an average of 22%) ($p < 0.001$) and qualitative indicators of ejaculate. The proportion of progressively motile sperm decreased by 11.3% at $p < 0.001$, of non-progressive motile sperm increased by 10.8% at $p < 0.01$. Sperm motility is one of the main parameters that characterize male fertility. Bull sires are used for reproduction depending on the spermatozoa motility [28]. Ejaculates with sperm motility values below 70% are rejected. The progressive movement of spermatozoa is necessary for them to reach the ovum. The absence or low content of sperm in the ejaculate with a straight-forward movement causes infertility [26, 28, 29].

The effect of geomagnetic disturbances is complex and depends both on their strength and the state of the biological objects themselves and their systems [24]. Analysis of the seminogram of bull sires in dynamics shows that the studied animals had decreased sperm production indicators during the days of geomagnetic activity. It should be noted that the reaction of bull sires to the geomagnetic situation was individual. The decrease in the number of progressively motile spermatozoa in individuals ranged from 11 to 16% ($p < 0.05$).

Numerous studies confirm the influence of abiotic factors on spermatozoa both when exposed to such agents on the paternal body, and in ejaculates or when found in the female tract [21-23]. Changes occur in the general morphology, the state of the nucleus and acrosome, in the mitochondria, filament, and other structures of spermatozoa [24, 25]. We observed an increase (by 40%, $p < 0.05$) in the proportion of sperm with abnormal morphology during the period of geomagnetic activity. At the same time, the share of spermatozoa with filament pathology increased the most (by 40.1%, $p < 0.05$).

The integrity of nDNA in the sperm chromatin is the most important characteristic of the biological full-value of spermatozoa [30, 31], it also determines fertility, the effectiveness of ovum fertilization and embryo development [32]. This study confirmed an increase in the index of DNA fragmentation in spermatozoa from ejaculates collected during the days of geomagnetic disturbances. The fragmentation degree varied from 1.80 to 38.84%, also indicating that the response to geomagnetic activity depends on the individual characteristics of the organism to a certain extent. It is known that one of the factors that cause DNA fragmentation in spermatozoa chromatin is oxidative stress [32, 33]. The plasma membrane of the spermatozoa contains a large amount of polyunsaturated fatty acids, which makes these germ cells more sensitive to such stress.

Thus, the data obtained in the present study indicate the influence of geomagnetic activity on the qualitative and quantitative characteristics of bull sperm obtained during the days of the magnetic storm. Comparative analysis of sperm biological full-value indicates an increase in the frequency of morphological anomalies and an increase in the index of nuclear DNA fragmentation in sperm from such ejaculates. These data obtained in vivo are important for the practice of using reproductive technologies. The observed effects should be considered as a result of the magnetic field influence on the organism level. A more detailed understanding of the processes occurring, in this case, can be obtained by an in-depth study of the physiological status of animals and the functional activity of the body systems, primarily reproductive and neurohumoral. Experiments with ejaculated cattle spermatozoa in vitro will be continued to fundamentally study the molecular mechanisms involved in these interactions and provide a cellular response to the action of geomagnetic fields.

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