

Original Research Paper

# Biotic and Abiotic Factors of Sperm nDNA Fragmentation in Farm Animals

<sup>1</sup>Baylar Iolchiev, <sup>1</sup>Natalya Volkova, <sup>1</sup>Pavel Klenovitskiy, <sup>1</sup>Vugar Bagirov, <sup>2</sup>Neilia Khusnetdinova, <sup>1</sup>Prytkov Yuri, <sup>3</sup>Anna Tadzheva and <sup>1</sup>Anastasia Silanteva

<sup>1</sup>Federal Research Center for Animal Husbandry Named after Academy Member L.K. Ernst, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia

<sup>2</sup>Skryabin, Academy of Veterinary Medicine and Biotechnology, 23, Scryabin Str., 109472 Moscow, Russia

<sup>3</sup>Peoples' Friendship University of Russia (RUDN University), 6, Miklukho-Maklaya Str., Moscow, 117198, Russia

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## Corresponding Author:

Baylar Iolchiev

Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia  
Email: baylar1@yandex.ru

**Abstract:** Infertility and subfertility are among the global challenges of our time. Fertility in livestock farming is not only biologically important, it is also economically important. The aim of this study was to study the effect of these factors on sperm chromatin nucleus and to assess the impact of the fragmentation index degree on bull reproductive performance. The influence of abiotic factors such as ambient temperature, the level of geomagnetic activity and the biotic factor-infectious diseases of male reproductive organs - was studied. At high ambient temperatures (28-30°C), the proportion of sperm with damaged DNA increased by 70% compared to temperatures below 15°C. A Multivariate Analysis Of Variance (MANOVA) confirmed the influence of the geomagnetic activity factor on the degree of nuclear DNA fragmentation in bull sperm cells (level  $p < 0.05$ ). During the summer period, on days with increased geomagnetic activity, the Nuclear DNA fragmentation index in the samples studied was 20.5%. In some of the samples studied, this index exceeded 38% and the coefficient of variation for this index reached 44%. The proportion of sperm cells with abnormal movement also increased during geomagnetic activity. The content of such sperm cells reached 9.1% in bull semen ejaculates obtained during the winter period. More than 12% of sperm had non-progressive movement in summer semen obtained with a K-index  $\geq 5.0$ , which was 83.8% higher than with a K-index  $\leq 1.0$ . High geomagnetic activity and temperature and infection of the reproductive organs lead to pathological changes in spermatozoa, an increase in the proportion of spermatozoa with damaged nuclear DNA and a decrease in fertility.

**Keywords:** Bulls, Fertility, DNA Fragmentation, Geomagnetic Activity, Ram, Semen

## Introduction

The main function of sperm cells is not only to deliver male genetic material to the egg, its fertilization and stimulation of embryo development, but also to ensure the development of a complete embryo (Li and Lloyd, 2020; Aitken *et al.*, 2013). Fertility is one of the global problems of our time, more than 15% of married couples around the world face this problem, it is also characteristic of animal breeding, about 25% of bulls are subfertile or infertile (Kim, 2018). The diagnosis of male

fertility is based on classical methods of analysis, in accordance with the criteria of the World Health Organisation (Santi *et al.*, 2018). The fertility tests used do not provide complete information on the biological completeness of sperm cells and do not allow the evaluation of all structural units. The prognosis of fertility and the results of the use of assisted reproductive technologies based on such tests cannot be highly accurate and they do not allow differentiation between fertile and subfertile individuals (Björndahl *et al.*, 2016; Cho and Agarwal, 2018). Additional tests are used to

assess the fertility of males. Special attention is paid to the condition of genetic material in sperm in order to determine their competence (Shamsi *et al.*, 2011). Sperm nuclear DNA fragmentation and denaturation has a negative impact on their fertility, embryo development and may also cause idiopathic infertility (Agarwal and Said, 2003). In subfertile male individuals, the DNA fragmentation index is higher than in fertile individuals. Fertilization of sperm oocytes with damaged chromatin DNA may be a cause of genetic disease in offspring (Zini *et al.*, 2001). Modern assisted reproductive technology makes it possible to successfully fertilize oocytes with sperm with low biological integrity, which raises questions about the feasibility of performing DNA fragmentation tests in sperm chromatin (Twigg *et al.*, 1998). Numerous studies have been conducted on the relationship between fertility and DNA fragmentation in sperm cells (Osman *et al.*, 2015; Choi *et al.*, 2017). The methods and protocols of analysis differ significantly, which is the reason why there is a lack of reliable information about the impact of genetic material fragmentation in sperm DNA on male fertility (Simon *et al.*, 2017; Deng *et al.*, 2019; Liang *et al.*, 2019). Determining the fragmentation index to predict fertility is important, including for animals (Ortiz *et al.*, 2017; Evenson, 2016; Esteves, 2016; Ozkosem *et al.*, 2015). A high fragmentation index has a negative impact on reproductive performance not only when artificial insemination or other assisted reproductive techniques are used, but also when conception is natural (Zini, 2011). Studies show that if the fragmentation index is higher than 30%, natural conception is not possible (Evenson *et al.*, 2002). High levels of sperm DNA fragmentation can lead to early abortion of pregnancy (Carrell *et al.*, 2003). In order to achieve high performance, sperm cell with intact DNA must be used for fertilization using assisted reproductive technology. This is especially important for subfertile individuals. The use of sperm with intact DNA is also necessary for the cryopreservation, as they are subjected to different technological influences (Kumar *et al.*, 2019; Lusignan *et al.*, 2018). The appearance of various DNA damage may be due to the age of individuals, infectious diseases, chronic or acute inflammatory disorders, environmental pollution, temperature stress, irradiation, incomplete apoptosis, as well as sperm storage conditions, technology for freezing and thawing sperm (Cankut *et al.*, 2019; Evgeni *et al.*, 2015). One of the main factors causing sperm DNA damage is male genital tract infection (Askienazy-Elbhar, 2005). The products of infected cells contain active forms of oxygen, which cause damage to the DNA structure and result in chromatin ruptures (Sakkas and Alvarez, 2010). The DNA fragmentation index of sperm cells depends on the method of obtaining them. Male sex cells extracted from the epidermis, compared to

ejaculated sperm, have a low chromatin DNA fragmentation index (Esteves *et al.*, 2015). Cryopreservation of generative plasma, including sperm, is widely used in reproductive technology. Various methods and protocols are used for cryo-preservation of sperm cells, which differ in the rate at which the bio-material is cooled and therefore they differ in the degree of DNA damage in sperm cells. Studies show that the smallest fragmentation index is found when using vitrification (Agha-Rahimi *et al.*, 2014; Isachenko *et al.*, 2019; Li *et al.*, 2019). One of the natural abiotic factors affecting biological processes and living organisms is geomagnetic activity (Binhi and Prato, 2017; Begall *et al.*, 2008). The mechanisms of magnetic storm influence on biological processes have not yet been fully studied, but the relationship of most physiological rhythms to geomagnetic activity is an undeniable fact (McCraty *et al.*, 2017). High geomagnetic activity may lead to the disruption of transmission movement and reduce the buffer capacity of the antioxidant system. Some structural units in cells, such as mitochondria and endoplasmic reticulum, are more sensitive to magnetic fields (Glinka *et al.*, 2018). Electromagnetic fields have a significant influence on sperm motility and morphology (Li *et al.*, 2010; Kumari *et al.*, 2017). The study sperm quality problem is of great economic and biological importance, as the development of cryopreservation, artificial insemination and other assisted reproductive technologies opens up the possibility of inseminating the semen of one male of several tens of thousands of females. The use of poor quality semen can lead to many million losses. Reproductive indicators, including semen quality, depend on numerous exogenous and endogenous factors. The aim of this research was to study the influence of biotic and abiotic factors on the state of chromatin nuclear DNA in farm animals.

## Materials and Methods

### Legal Requirements

The protocols of experiments were approved by the Committee for Animal Care and Use of the L.K. Ernst Federal Research Centre for Animal Production in accordance with Decision No. 80 of the Council of the Eurasian Economic Commission of 10 November 2017 "On Approval of the Rules for Organization of Laboratory Testing during Veterinary Control (Supervision)".

### Animals and Diagram of Research

The objects of research were Romanov rams (n = 10), Romanov sheep and argali hybrids (n = 7) and bulls (n = 78) of different breeds and directions of productivity (Table 1). The test animals were clinically healthy. We studied the effect of the nDNA fragmentation index on sperm motility on the state of

acrosomes, the proportion of pathological forms of spermatozoa. The influence of ambient temperature and geomagnetic activity on the index of nDNA fragmentation of spermatozoa, on their activity, morphology and acrosome was studied. During the study period, the ambient temperature ranged from -13 to +30C. The K-index characterizing the geomagnetic activity varied from 0 to 5. To study the effect of a disease of the reproductive tract on the level of fragmentation, the quality of sperm of bulls diagnosed with balanoposthitis was studied. The diagnosis of animal disease was confirmed by PCR analysis.

2.3. *Monitoring of geomagnetic activity* was conducted according to the Institute of Earth Magnetism, ionosphere and radio wave propagation. N.V. Pushkova, Russian Academy of Sciences (<http://geodata.izmiran.ru/>).

### Collection of Semen

Semen from bulls was collected in a truncated artificial vagina with a disposable sterile polyethylene sperm receiver. Two ejaculates were taken from each bull producer with an interval of 10-15 min. The ejaculates were diluted with the OptiXcell synthetic medium depending on their concentration. Cryopreservation of bull semen was carried out in straws.

Semen from rams was collected in an artificial vagina. "OVIXCell" (IMV TECHNOLOGIES) was used to dilute semen.

### Semen Analysis

The collected ejaculate were evaluated on quantitative and qualitative indicators. The volume of obtained semen was determined.

### Sperm Motility

Argus-CASA software was used to evaluate sperm activity. The sperm Velocity rates measured included the Curvilinear (VCL), Straight Line (VSL) and Average Path Velocity (VAP), linearity (LIN), Straightness (STR), wobble (WOB), Amplitude of Lateral Head displacement (ALH), Beat Cross Frequency (BCF) and Hyperactivity (HPA). Sperm, depending on the parameters, the movement is divided into classes: PR-Progressive Mobility; NP- Non-Progressive mobility and IM-Immobility.

### DNA Sperm Fragmentation

DNA sperm fragmentation was studied using the Halosperm® kit (INDAS laboratories, Madrid, Spain) in accordance with the protocol. The semen samples were diluted to a concentration of 20 million sperm per ml. Then, spermatozoa were immersed in agarose microgel and spread on the slide. Samples were denatured with an acid and lysis solution, dehydrated and stained with Diffquick. Sperm with large halos (thicknesses that were similar or larger than the length of the smallest diameter of the core) and sperm with medium-sized halos (thickness greater than 1/3 of the smallest diameter of the core and less than the smallest diameter of the core) were classified as 'spermatozoa having no fragmentation.

### Analysis Morphology and Acrosome

Studying the condition of acrosome. The acrosomal integrity was studied using the Diahim-Diff-Quick differential staining (Borunova *et al.*, 2017).

Sperm morphology was studied using electron microscopy (Bragina and Bocharova, 2014). Sperm morphology was classified according to the Krueger criterion.

### Statistical Analysis

Analysis Of Variance (ANOVA) was used to determine the influence of the ambient temperature factor on the ram sperm DNA fragmentation index. The same analysis method was used to determine the effect of nuclear DNA fragmentation index on sperm motility and morphology of individual germ cell segments. ANOVA was used to evaluate the impact of bull reproductive disease on sperm DNA status. Multivariate Analysis Of Variance (MANOVA) was used to determine the impact of geomagnetic activity level (K-index) and ambient temperature in different seasons on sperm DNA fragmentation. We studied the effect of these fixed factors on the following variables: Sperm motility, content of pathological forms of sperm, sperm with intact acrosomes. The Scheffe's multiple comparison method was used to identify differences between groups. At  $p < 0.001$  the differences were considered statistically highly significant, at  $p < 0.01$  and  $p < 0.05$  the differences were considered statistically significant.

**Table 1:** Study design

Species	n	Fixed factor	Fixed factor affecting DFI	A research variable
<i>Bos taurus taurus</i>	37	nDNA fragmentation index in spermatozoa	-	Sperm motility, the content of pathological forms of spermatozoa, sperm count with intact acrosome, bovine reproductive index
<i>Ovisaries</i>	17		Ambient Temperature	Sperm nDNA fragmentation index, sperm motility,
<i>Bos taurus taurus</i>	35		Geomagnetic activity and temperature	the content of pathological forms of spermatozoa, sperm with intactacrosome
<i>Bos taurus taurus</i>	4	nDNA fragmentation index in spermatozoa	Reproductive Tract infection	Sperm DNA fragmentation index, sperm motility, the content of pathological forms of spermatozoa, sperm count with intact acrosome, bovine reproductive index

## Results

### *Effect of Environmental Temperature on Semen Quality*

During the summer period, when the ambient temperature rises to 28-30°C, the content of motile sperm in freshly obtained ram semen reached 74.3±3.6% and the proportion of sperm with pathological morphology did not exceed 2.6%. The sperm DNA fragmentation index in this case was 10.8±1.3% (Table 2).

The drop in ambient temperature to 15°C and below in autumn was accompanied by an improvement in the biological integrity of the ram's sperm cells. The content of motile sperm in the semen increased by 16.7% ( $p<0.01$ ) compared to the summer period. There was a decrease in the proportion of sperm cells with pathological morphology from 2.6 to 1.9% ( $p<0.001$ ).

Semen obtained during the summer and autumn periods was used for cryopreservation with subsequent assessment of biological integrity of frozen-thawed semen. The content of progressively motile sperm in summer ejaculate samples was 36.0% and in autumn it was 43.6% (Table 3). The freeze-thaw cycle of semen

was accompanied by an increase in the proportion of sperm with damaged nuclear DNA compared to newly obtained semen. In the autumn semen samples, the proportion of such sperm cells was 85% lower than in summer ( $p<0.001$ ).

### *Relationship between Reproductive Performance and the Degree of Sperm Nuclear DNA Fragmentation*

The results of study on the integrity of frozen thawed bull semen showed that 18.9% of male DFI reached 15%. In 8.1% of the examined individuals, this figure exceeded 50% (Table 4). The content of progressively motile semen in the studied ejaculates varied depending on the established DFI values. The proportion of PR sperm cells in semen samples with DFI below 15% was 44.6%. With DFI of 16-49%, this index decreased by 7.3% ( $p<0.001$ ), with DFI over 50% by 12.5% ( $p<0.001$ ). A high rate of cow pregnancy from the first insemination was found when bull semen with DFI of less than 15% was used. This indicator reached 47%. With the use of sperm DFI semen 16-49%, the insemination efficiency of cows was reduced by 15.8% ( $p<0.001$ ), with the use of DFI semen over 50% - by 13% ( $p<0.001$ ).

**Table 2:** Impact of environmental temperature on ram semen quality

Dependent variable	Observations, <i>n</i>	Ambient temperature	
		$t \geq 28-30^\circ\text{C}$	$t \leq 15^\circ\text{C}$
Animals, <i>n</i>		17	17
Volume, mL	170	1.35±0.1	1.15±0.1
Sperm motility, %	170	74.3±3.6	86.7±2.5**
Abnormal sperm morphology, %	170	2.6±0.1	1.9±0.1***
DNA fragmentation index (DFI), %	170	10.8±1.3	6.2±0.8**
Sperm with intact acrosome, %	170	86.3±2.5	92.2±3.6

\* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$

**Table 3:** Quality of frozen and thawed semen obtained at different temperature conditions

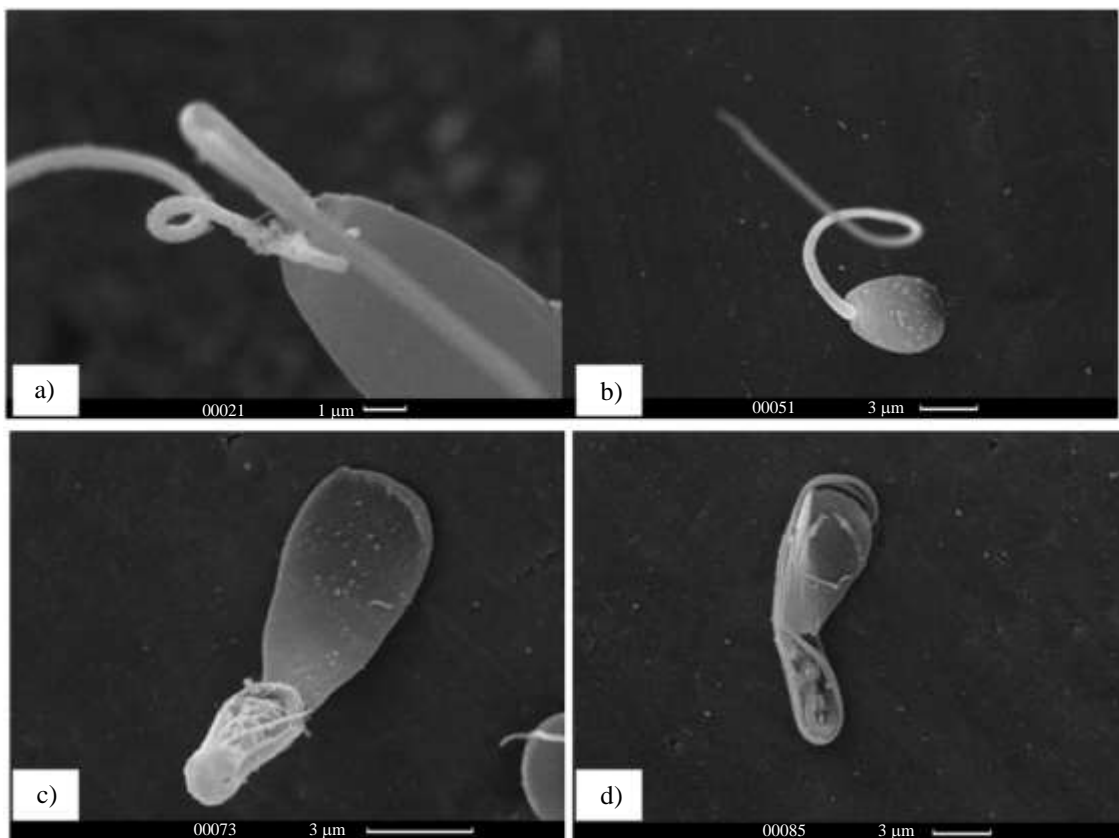
Dependent variable	Observations, <i>n</i>	Ambient temperature	
		$t \geq 28-30^\circ\text{C}$	$t \leq 15^\circ$
PR, %	170	36.0±1.8	43.6±1.5**
NP, %	170	12.6±1.5	5.7±0.9**
IM, %	170	51.4±1.7	50.7±1.1
Head pathology, %	170	50.6 ±1.6	46.1±0.8*
Pathology of the middle part, %	170	11.9±0.4	12.3±0.3
Flagellum pathology, %	170	37.5±1.3	41.6±1.6*
Sperm with damagedn DNA, %	170	21.3±0.6	11.5±0.8***

PR -progressive motility; NP - non-progressive motility; IM- immobility

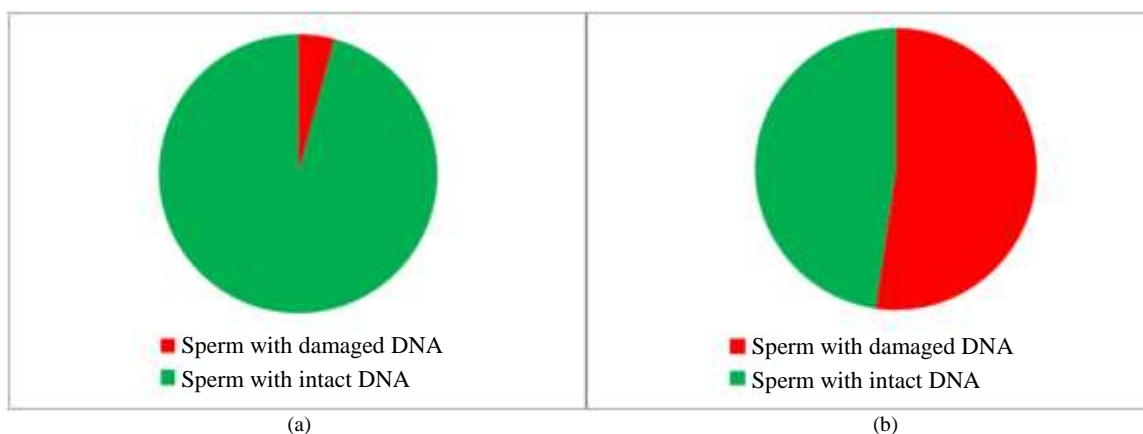
**Table 4:** Impact of fragmentation index of nuclear DNA on reproductive efficiency of bulls

Dependent variable	DFI<15%	DFI>16-49%	DFI>50%
Animals, <i>n</i>	30	4	3
DFI,%	2.2±	27.8±1.6***	72.04±3.6***
Sperm motility, %	44.6±1.4	37.2±1.7***	32.1±2.6***
Pregnancy, %	52.0±2.3	36.0±1.4***	28.3±1.6***
Pregnancy from the first insemination, %	47.0±1.2	31.2±0.9***	24.0±0.8***

\* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; High geomagnetic activity and temperature and infection of the reproductive organs lead to pathological changes in spermatozoa, an increase in the proportion of spermatozoa with damaged nuclear DNA and a decrease in fertility



**Fig. 1:** Electron microscopy of bull sperm with pathological morphology



**Fig. 2:** Impact of reproductive organ infection on bull sperm DNA fragmentation index; (a) clinically healthy bulls; (b) bulls with infectious diseases of reproductive organs

### *Effect of Geomagnetic Activity on Semen Quality*

The results of MANOVA have shown that the combination of environmental temperature factor and geomagnetic activity has a statistically significant effect on the activity and content of sperm cells with abnormal movement in the freshly received semen ( $p < 0.05$ ). The minimum low temperature on geomagnetic activity days

( $K\text{-index} \geq 5.0$ ) was observed in February and dropped to  $-13^{\circ}\text{C}$ . The proportion of sperm cell with pathological morphology in bull semen ejaculates obtained during this period was 7.8%. Bull semen ejaculates obtained in a period with similar low surrounding temperatures but no high geomagnetic activity were also studied. The content of sperm cell in semen with pathological morphology in this case decreased by a factor of 1.5 compared to the

period of high geomagnetic activity ( $p < 0.05$ ). More than 50% of the detected pathologies in the sperm cells were caused by flagella anomalies. The results of electron microscopy in some forms of bull sperm pathology on days of geomagnetic activity was showed on Fig. 1.

During geomagnetic activity, the proportion of sperm cells with abnormal movement also increased. In bull semen ejaculates obtained during the winter period, the content of such sperm was 9.1%. In summer semen obtained with a K-index  $\geq 5.0$ , more than 12% of sperm had retrogressive movement, which was 83.8% higher than with a K-index  $\leq 1.0$ .

### *Influence of Bull Reproductive Organ Infections on Sperm Nuclear DNA Fragmentation Index*

One of the main biotic factors affecting the reproductive performance of male and female individuals is infectious diseases of the reproductive organs. The study results showed that the sperm DNA fragmentation index in clinically healthy bulls was  $4.2 \pm 1.3\%$ . With infectious diseases of reproductive organs in these bulls, this index increased to  $52.3 \pm 4.17\%$  (Fig. 2).

## **Discussion**

For the first time, a comprehensive study of the impact of biotic and abiotic factors on nuclear DNA fragmentation in sperm cells in farm animals was conducted. The influence of surrounding temperature as an abiotic factor on the degree of fragmentation of ram sperm DNA has been studied and the relationship between sperm quality and the degree of fragmentation of chromatin DNA has been analyzed. Data on the influence of ambient temperature on ram sperm quality are consistent with data from (Belkadi *et al.*, 2017; Santos *et al.*, 2015). High ambient temperatures can lead to disruption of individual sperm cells, reducing their biological integrity and to complete or partial infertility (Salces-Ortiz *et al.*, 2015). The results of our research have shown that the proportion of sperm cells with damaged nuclear DNA increases in ram semen under high ambient temperatures. The our obtained data about the negative impact of high temperature on DNA status corresponds to those obtained by (Pérez-Crespo *et al.*, 2008). The negative effect of high temperature on sperm DNA integrity has been confirmed in other mammal species, such as mice (Fleming *et al.* 2004). An increase in temperature reduces the synthesis of the protein covering the sperm membrane, which, in turn, leads to the formation of morphologically abnormal spermatozoa. Sertoli cells are essential in spermatogenesis. High temperature damages Sertoli cells, reduces their number and disrupts the process of spermatogenesis. One of the damaging effects of high temperature is the activation of p53, which causes the cell cycle to stop. The high

temperature of the scrotum cause condensation of nuclear chromatin. A number of studies have shown that one of the important abiotic factors affecting biological objects is geomagnetic activity (Binhi and Prato, 2017; McCraty *et al.*, 2017). The results of MANOVA show that high geomagnetic activity leads to an increase in DNA fragmentation index in animal sperm. The influence of geomagnetic activity at the cellular level is explained by changes in the state and functions of cell membranes, a violation of transmembrane transport, the formation of free radical lipid oxidation products and a decrease in the buffer capacity of the antioxidant system. As a result of this process, reactive oxygen species are formed, which are one of the main factors causing nuclear DNA fragmentation in spermatozoa. In studies (Evenson *et al.*, 2002) it has been established that natural conception is not possible with a sperm DNA fragmentation index of more than 30%. The results of our studies show that cows can be fertilized with 50% of bull sperm DNA damage. It should be noted that the effectiveness of artificial insemination in this case was reduced by a factor of 2 and did not exceed 25%. Inflammatory processes in infections of the reproductive organs lead to oxidative stress, which leads to an increase in the index of DNA fragmentation. Infection of the reproductive organs of bulls also increased the proportion of sperm with damaged DNA from 4.17 to 52.3%.

## **Conclusion**

The results of a study of the impact of various abiotic and biotic factors on the sperm quality of farm animals showed that there is a correlation between the sperm nuclear DNA fragmentation index and exogenous factors such as the surrounding temperature and geomagnetic activity. High geomagnetic activity results in an increased proportion of sperm with damaged nuclear DNA. During the period of high geomagnetic activity, there was a decrease in sperm movement and an increase in sperm content with abnormal morphology in the received bull semen ejaculates. Pathological changes in the flagellum area were most frequently observed. Infectious diseases of the male reproductive organs also contributed to sperm cell damage. An increase in the nuclear DNA fragmentation index resulted in a decrease in fertility.

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## **Author Contributions**

All authors have read and agreed to the published version of the manuscript.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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