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EFFECT OF ATMOSPHERIC PRESSURE ON SEMEN PARAMETERS IN BULL SIRE OF MODERN SELECTION ON THE DAY OF COLLECTION

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Abstract

The publications concerning the effects of atmospheric pressure on the general patterns of metabolism, reproduction and adaptive capability in animals and humans from 1970 to 2015 are reviewed. The analysis could show that all those investigations were carried out on different mammalia species in different years and were fragmentary. Colossal changes in the Earth's atmosphere occurred over the recent decades, which were caused by the anthropogenic factors; considerable changes in the environment affected the habitats of living organisms. In addition, the rapid development in the livestock farming on the basis of the accelerated selection processes could contribute to the formation of animal breeds characterized by the changed metabolic functions, the affected stress tolerance, and the lower adaptive capabilities. That enabled us to conduct a survey to ascertain the effect of atmospheric pressure variability on the qualitative and quantitative semen parameters for bull sires of a modern selection. The survey was carried out in the Center for Animal Biotechnology and Molecular Diagnostics, the L.K. Ernst All-Russia Research Institute for Animal Husbandry, on the basis of the Head Center for Reproduction of Farm Animals. The collection, evaluation, freezing, thawing, and the use of the pedigree bull sire semen were carried out according to the National Technology (edited by N.M. Reshetnikov and A.I. Abilov, 2008). A total of 472 ejaculates of bull sires aged 2 to 10 years were analyzed in January, 2012. The atmospheric pressure varied in that period as follows: lower 755 mm Hg, from 755 mm Hg to 765 mm Hg, and over 765 mm during the periods of 7 days, 9 days, and 15 days, respectively. The ejaculate volume (ml), concentration (million per ml) and motility of spermatozoa with the progressive forward movements (PFM) (grades) were analyzed after the collection, freezing, thawing, and in 5 hours of incubation at 38 °C; the total number of spermatozoa per ejaculate (million), sperm defects (%), number of frozen semen doses per ejaculate, and the supposed loss of the semen doses caused by the sperm defects depending on both the variables of atmospheric pressure and the age of animals were examined comparatively. The obtained data were processed; the statistical reliability was calculated by the Student's *t*-criterion. It is ascertained that the atmospheric pressure substantially affects the quantity and the quality of the obtained sperm products. These effects can be neatly traced by the number of sperm defects at sampling with defining a statistically valid value ($P > 0.001$). The bull sires aged 2.5 to 5 years are found to be more adaptive to the effects of various environmental factors. It is determined that the culling rate of the native semen samples by the activity parameter decreases with increasing the atmospheric pressure. The distinct dependency of the decrease in motility of sperm after the incubation at 38 °C from the increase in the atmospheric pressure was revealed. The best value for the number of the extracted spermatozoa was obtained at 760 mm Hg for each of the animal age groups; the lowest number of ejaculates having more than 5 million sperms was obtained at 775 mm Hg. Thus, the obtained data allow conclusion about the effects of atmospheric pressure variables on the sperm product parameters. In this case, the age of bull sires is of considerable importance. It is found that the bulls at the age of 2.5-5 years are more adaptive to the effects of various environmental factors. On the basis of the findings, it should be concluded therefore that in the breeding enterprises, the schedule for collecting the semen from the bull sires has to be corrected ac-

ording to the atmospheric pressure values. The use of bull sires should be limited or the schedule of their exploitation should be shifted at the atmospheric pressure of 775 mm Hg. The scientific novelty of the paper is in that the complex survey focused on the effects of atmospheric pressure variables on the qualitative and quantitative characteristics of the semen parameters in bull sires of the modern selection have been carried out for the first time.

Keywords: atmospheric pressure, bull sires, semen, age

In Russia, collection and use of semen of bull sires are regulated by the national technology [1], which currently does not consider the influence of a number of external factors on sperm production. At the same time, in many regions of the Russian Federation, including the Central Black Earth Region, due to long-term anthropogenic impact, an ecological situation is characterized by anthropogenic anomalies of different origin [2, 3]. Anthropogenic factors are related to realization of genetic potential in farm animals, including the inhibition of reproductive function in highly productive individuals, and a reduction in resistance to diseases and stresses. The adverse effects of environmental stress can occur at the genetic level. The precise identification of environmental factors affecting semen quality is important for improving bull reproductive function [4-6]. Morphofunctional abnormalities of the reproductive organs in cattle in a sharply continental climate reduce the reproductive capacity [7]. The season of the year affects the indicators of sperm production [8-13] and the proportion of cells with normal morphology due to changes in the hormonal background and the effects of ambient temperature [14, 15]. Winter and spring seasons are most favorable for sperm donation. In the summer period, stud bulls are relatively low sexually active. The total average monthly volume of sperm production indicates a decrease in activity in the autumn period, too [9, 16, 17]. The highest rates of ejaculate volume, semen count and sperm doses in spring are explained by photoperiodism and androgenic activity of bulls [10, 18]. To reduce the adverse effect of summer season factors on reproduction, it is proposed to reduce the frequency of semen taking and ensure better processing during cryopreservation [9].

In some studies, there was a positive correlation between atmospheric pressure and the birth rate of bull-calves [19]. There is evidence of the effect of high pressure on the sex ratio after insemination [20]. The effect of atmospheric pressure on the sex ratio was studied in Ecuador. Of 45 inseminated Holstein cows at an altitude of 2,750 m above sea level, 68.40 % have calved; 78.69 % of the calves from the heifers were also bull-calves ($P < 0.5$) [21]. In another experiment, 13 calves were born from cows inseminated with sperm which was exposed to reduced atmospheric pressure; 84.60 % of the calves were bull-calves. Of 12 calves born from cows inseminated with unprocessed sperm, 58.30 % were the bull-calves [21]. At 751.0-760.0 mm Hg atmospheric pressure, the time of manifestation of the copulation reflex in boars is much shorter than that at a higher or lower pressure, and the copulation and ejaculation reflex is longer. Atmospheric pressure exerted a significant influence on the biological indicators of boar semen quality. A larger volume of ejaculate and a higher concentration, an increase in resistance and an absolute survival rate of spermatozoa were noted in boars at 756 mm Hg and more [22]. It is reported that the sperm production of bulls was significantly influenced by atmospheric pressure and air humidity (along with other environmental factors) depending on animal breed, pedigree and age [23].

Sudden rain and strong winds act as negative factors in combination with low temperature, and solar radiation is unfavorable at high temperature [24]. Atmospheric pressure, solar activity, intensity of the Earth's magnetic field affect various physiological and biochemical indices of animals, e.g. the content of calcium, phosphorus, reserve alkalinity, carotene, and protein in the blood serum.

A significant decrease in dry matter digestibility was found in 58-83 % of cows [25]. If the atmospheric pressure deviates by 18-20 mm Hg from the mean normative index (750 mm Hg) and other conditions are equal, the enzymatic status of lymphocytes significantly changed [26]. In Hereford and Simmental cattle, feed intake increased with increasing atmospheric pressure [27]. There are data on the effect of atmospheric pressure on the duration of fruiting in cows and pigs [28]. When the intensity of abnormal meteorological factors over a long time exceeds physiological standards, the body's response becomes pathological, and an imbalance of the homeostasis is developed. Stresses result in a general adaptation syndrome, leading to hematological, morphological and clinical changes. Free radicals are intensively formed, oxidative stress and pathological processes develop [29, 30].

Protection of productive animals from impacts that disrupt the reproductive function and adversely affect growth and productivity are often associated with unprofitable costs. Therefore, study of sperm biochemical composition, the quality and fertilizing capacity of spermatozoa, as influenced by environmental conditions, age, breed and individual characteristics of the sire is relevant in improving reproduction [21].

In the present work, we conducted for the first time a comprehensive study of the effect of high atmospheric pressure over a long period on the sperm production in bull sires depending on their age.

Our goal was to assess the qualitative and quantitative indicators of semen in bull sires at different atmospheric pressures.

Technique. Semen from 35 Holstein bull sires aged from 2 to 10 years (breeding herd, OAO Head Center for the reproduction of farm animals, Moscow region, 2012) was collected in an artificial vagina with a doublet mounting with 10-15 min interval. The measured sperm parameters were the volume of ejaculate (ml), the concentration (bn/ml) and the motility (%) of native spermatozoa in the semen, the total number of spermatozoa in the ejaculate (bn), the number and percentage of the discarded semen, the rectilinear motility of the spermatozoa after freezing-thawing and after 5 hours of incubation at 38 °C (1). The main criteria for the semen quality were the number of frozen semen doses per ejaculate. All technological regulations were carried out in accordance with the national technology of freezing and using semen of breeding bull sires [1].

The atmospheric pressure records were obtained from meteorological service [http://rp5.ru/Погода_в_Москве_\(юг\)](http://rp5.ru/Погода_в_Москве_(юг)). The dates of the experiment were combined into conditional groups, i.e. with a pressure of < 744.9 mm Hg (1 day), 745.0-754.9 mm Hg (6 days), 755.0-764.9 mm Hg (9 days), 765.0-774.9 mm Hg (7 days), and > 775.0 mm Hg (8 days).

The data was processed in the Microsoft Excel program with confirmation of reliability by the Student's *t*-test. The mean (*M*), minimum and maximum values (min-max) of indicators, mean errors (*m*), and the levels of statistical significance of the differences (*P*) are presented.

Results. In total, 530 ejaculates were studied during the experiment, 132 of them were rejected as not meeting the technological requirements [1]. The greatest number of ejaculates was obtained at high atmospheric pressure (Table 1). It should be noted that a significant (2.5-3.0-fold, *P* < 0.001) decrease in culled out native semen occurred when atmospheric pressure was the highest, i.e. 755.0-764.9 mm Hg (9 days) and 775.0 mm Hg (8 days).

In young bull sires aged 1.5 to 2.0 years, a decrease in atmospheric pressure caused hypoxia which explains the significant amount (up to 50 %) of culled ejaculates (Table 2). With the increase in atmospheric pressure, the proportion of culled ejaculates lowered significantly.

1. Atmospheric pressure to the dates of the experiment and the rejection of native semen obtained from Holstein bull sires (Moscow region, January 2012)

Atmospheric pressure, mm Hg		Examined ejaculates		
min-max	$M \pm m$	total	culled	
			number	% ($M \pm m$)
< 744.9 (1 day)	744.0	46	13	28.26±4.69**
745.0-754.9 (6 days)	752.00±0.08	28	11	39.29±6.53
755.0-764.9 (9 days)	761.20±0.81**	57	31	54.39±4.66*
765.0-774.9 (7 days)	769.00±0.85**	188	40	21.28±2.11***
> 775.0 (8 days)	784.70±1.77*	213	37	17.37±1.84***

*, ** and *** mean $P < 0.1$, $P < 0.01$ and $P < 0.001$, respectively, as compared to 745.0-754.9 mm Hg

2. The percentage of the primary culled semen of Holstein bull sires of different age depending on the atmospheric pressure on the date of ejaculate collection ($M \pm m$, Moscow region, January 2012)

Age	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	42.31±6.85	50.00±17.68	33.33±4.81	16.87±2.91**	19.81±2.74*
2.5-5.0 years	0	0	35.29±8.20	25.00±12.10	12.90±4.26*
6.0 years and older	16.67±10.76	42.86±9.35	40.74±6.69	23.68±4.88*	0

*, ** means $P < 0.05$ and $P < 0.01$, respectively, between atmospheric pressure gradations.

The bulls aged 2.5 to 5.0 years were less dependent on atmospheric pressure which, in our opinion, is due to adaptation mechanisms. At a relatively low atmospheric pressure (< 755.0 mm Hg), the semen of these bulls was not rejected, but due to the lack of ejaculates, it is difficult to estimate the reliability of the results. In sires aged 6 years and older, with an increase in atmospheric pressure, a significant decrease in the number of culled ejaculates was also noted.

3. Activity (%) of spermatozoa with rectilinear motility in the frozen-thawed semen of Holstein bulls of different age after 5 hours of incubation (at 38 °C) depending on atmospheric pressure on the date of ejaculate collection ($M \pm m$, Moscow Region, January 2012)

Возраст	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	18.5±10.5	Нет данных	17.22±12.20	13.66±6.50	16.81±9.10
2.5-5.0 years	23.33±5.80	18.33±10.40	15.00±7.10	15.91±8.90	14.17±6.40
6.0 years and older	21.67±17.60	21.00±7.40	13.12±11.90	12.00±8.10	16.92±9.50

As per the RF State Standard (GOST 26030-83) for frozen semen of bulls [1], after thawing all series of cryopreserved sperm that do not meet the requirements are culled, and the cryopreserved sperm with a mobility of 40 % and higher is stored. In our experiment, the motility of the semen immediately after thawing averaged 40.0-43.5 % (regardless of age and atmospheric pressure on the date of collection). Samples that contained less than 40 % of spermatozoa with rectilinear motility after thawing were discarded and their results was not considered. Samples that meet the quality standards were taken for subsequent incubation.

Further, the viability of the thawed semen was studied after 5-hour incubation at 38 °C (Table 3). Quality was maintained in 18.5-23.3 % of spermatozoa ejaculated at low atmospheric pressure, and in 12.0-17.0 % of those obtained at 765.0-774.5 mm Hg. That is, with an increase in atmospheric pressure during ejaculation, the lifespan of cryopreserved spermatozoa during post-thawing incubation reduced.

There was a tendency to increase the loss of semen quality during incubation depending on atmospheric pressure (Table 4). Thus, for the semen obtained under low atmospheric pressure, losses during 5 hours at 38 °C were 44-48 %, reaching 50-54 % for 745.0-754.4 mm Hg, 58-67 % for 755.0-764.5 mm Hg, 61-71 % for 765.0-774.5 mm Hg and 58-65 % for > 775 mm Hg.

4. Loss of quality (%) in the frozen-thawed semen of Holstein bull sires of different age after 5 hours of incubation (at 38 °C) depending on atmospheric pressure on the date of ejaculate collection ($M\pm m$, Moscow region, January 2012 of the year)

Age	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	47.47±11.65	Нет данных	57.83±8.23	66.46±6.72	58.12±5.81
2.5-5.0 years	44.01±15.70	54.17±17.62	62.50±10.32	60.94±6.77	64.91±6.38
6.0 years and older	48.00±15.80	50.00±15.81	67.20±10.50	70.73±8.60	57.70±9.69

The greatest volume of ejaculates from bull sires was obtained at normal atmospheric pressure (755.0-764.5 mm Hg). The lowest volumes were at the peak of atmospheric pressure (784.7±1.77 mm Hg) (Table 5). The decrease in volumes was observed above the level of 765.0 mm Hg and did not depend on the age of the sires.

In young bulls at 755.0-764.5 mm Hg the ejaculate volume was 4.21±1.36 ml, and with an increase in atmospheric pressure for every 10 mm Hg this indicator decreased by 15-20 %. In animals of active reproductive age (2.5-5.0 years) at 765.0-774.5 and > 775.0 mm Hg the ejaculate volume decreased by 34.39 and 28.32 %, respectively. In bulls over 6.0 years of age, the reduction was 4 % at 765.0-774.5 mm Hg and 28.9 % at 775.0 mm Hg and higher.

5. Average volume of ejaculate (ml) in Holstein bull sires of different age depending on atmospheric pressure on the date of semen collection ($M\pm m$, Moscow region, January 2012)

Age	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	3.85±1.60	Нет данных	4.21±1.36	3.32±1.16	2.82±0.77
2.5-5.0 years	6.33±0.58	4.83±1.61	6.92±2.69	4.54±1.60	4.96±2.60
6.0 years and older	4.67±2.08	6.20±3.19	4.89±1.34	4.69±1.85	3.82±0.88
Averaged	4.45±1.94	6.00±2.46	4.87±1.76	4.14±1.60	3.42±1.51

6. The number of spermatozoa (bn/ml) in the semen of Holstein bull series of different age depending on the atmospheric pressure on the date of ejaculate collection ($M\pm m$, Moscow region, January 2012)

Age	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	1.18±0.24	No data	1.15±0.21	1.16±0.33	1.19±0.37
2.5-5.0 years	1.13±0.35	1.23±0.38	1.23±0.31	1.05±0.25	1.24±0.32
6.0 years and older	1.23±0.32	0.98±0.29	1.25±0.22	1.20±0.23	1.12±0.38
Averaged	1.18±0.24	0.94±0.24	1.21±0.23	1.16±0.31	1.20±0.36

The effect of atmospheric pressure on the number of spermatozoa in the semen was not significant (Table 6). However, its increase in bull sires aged 2.5-5.0 and 6.0 years was observed. At 755.0-764.5 mm Hg this was 1.23-1.25 bn/ml being 8-15 % higher than at other atmospheric pressure. As the pressure increased, there was a clear trend towards a decrease in the number of spermatozoa in the ejaculate. The results obtained at low atmospheric pressure, because of the small number of samples, made it impossible to draw an appropriate conclusion.

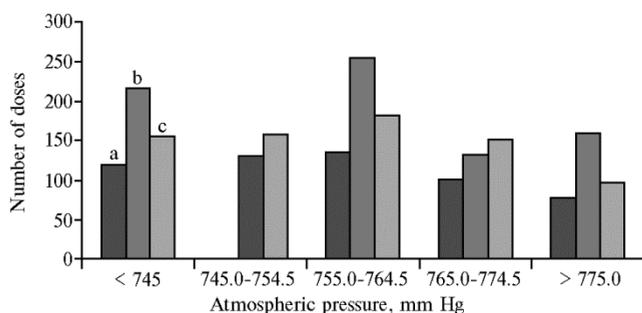
In young bulls still under the age of 2.0 years at 755.0-764.5 mm Hg the maximum number of spermatozoa (4.89 billion) was recorded in one ejaculate (Table 7). With an increase in atmospheric pressure, this indicator decreased by 22.7 % at 765.0-774.5 mm Hg, and by 33.95 % at < 775.0 mm Hg. In bulls aged 2.5-5.0 years, the greatest number of spermatozoa in the ejaculate (7.10 billion) was obtained at 755.0-764.5 mm Hg; this value decreased by 28 % at 769.0 mm Hg, and by 25 % at > 775.0 mm Hg. At low pressure, the reduction was 10-11 %. A similar trend was observed in bulls aged 6-11 years. The index was 6.02 billion at 755.0-764.5 mm Hg and decreased with an increase in atmospheric pressure to 765.0-774.5 and > 775.0 mm Hg by 8.5 and 32 %, respectively.

7. Number of spermatozoa (billion) in ejaculates of Holstein bull sires of different age depending on atmospheric pressure at the date of semen collection ($M \pm m$, Moscow region, January 2012)

Age	Atmospheric pressure, mm Hg				
	< 745	745.0-754.5	755.0-764.5	765.0-774.5	> 775.0
1.5-2.0 years	4.49±1.59	Нет данных	4.89±1.87	3.78±1.60	3.23±1.50
2.5-5.0 years	6.32±2.55	6.36±2.79	7.10±2.54	5.12±2.19	5.33±2.51
6.0 years and older	4.70±2.40	5.32±2.31	6.02±1.44	5.51±2.10	4.11±1.33

8. The percentage of ejaculates with sperm count > 5 billion in Holstein bull sires of different age depending on the atmospheric pressure on the date of semen collection ($M \pm m$, Moscow region, January 2012)

Age	Atmospheric pressure, mm Hg			Significance of the differences between the options		
	755.0-764.5 (I)	765.0-774.5 (II)	> 775.0 (III)	I и II	I и III	II и III
	1.5-2.0 years	52.63±8.10	27.80±5.28	9.30±3.13	P < 0.05	P < 0.001
2.5-5.0 years	90.90±6.13	46.15±6.91	54.10±6.66	P < 0.001	P < 0.001	P < 0.001
6.0 years and older	70.00±10.25	64.29±9.05	31.00±9.07	P < 0.05	P < 0.05	P < 0.05



The number of frozen semen doses per ejaculate in Holstein bull sires depending on the atmospheric pressure on the date of sperm collection: a, b, c — age of animals, 1.5-2.0 years, 2.5-5.0 years and over 6.0 years, respectively (Moscow region, January 2012).

with atmospheric pressure of 755.0-764.5 mm Hg (Fig.). At its increase to 765.0 mm Hg and higher the output of qualitative doses in all age groups decreased more sharply, i.e. by 43 % for young males, by 37 % for bulls of active reproductive age, and by 45 % for animals aged 6 years and older.

Thus, bull sires, due to their adaptive capabilities, are able to cope with short-term changes in atmospheric pressure. However, with prolonged exposure to exogenous factors, the body adaptation is aimed at self-preservation. As a result, the number of spermatozoa in the ejaculate decreases (e.g., in bulls over the age of 6.0 years this was 6.02 billion at atmospheric pressure 755-765 mm Hg and 4.11 billion at 775 mm Hg), and the volume of the semen decreases by 21-34 %. As a consequence, the yield of qualitative sperm doses per ejaculate decreases by 37-45 % in different age groups. Bulls of active reproductive age (2.5-5.0-year old) are most resistant to changes in atmospheric pressure. Based on the data obtained, it is necessary to correct the schedule for semen donation from bull sires with regard to atmospheric pressure. If during an extended period (8 days or more) the atmospheric pressure is kept above 775 mm Hg, the use of animals should be limited or the schedule of their use should be shifted.

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When atmospheric pressure increased, the number of ejaculates with a sperm count > 5 billion (Table 8) significantly decreased. This was well traced in young bulls and animals older than 6.0 years. The sires of the active reproductive age (2.5-5.0 years) turned out to be less weather-dependent.

The peak of sperm production in bull sires of all ages fell on the days

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