The aim of our study was to analyze the reproduction dynamics and fertility rates of the Ayrshire cattle population in Russia and to assess the level of influence of the AH1 haplotype on the incidence of stillbirth. For an overall fertility estimate, we used 390,769 records of 67 dairy herds in Russia from 2003 to 2018. Against the background of stable population growth, the average stillbirth rate was 3.33% and the abortion rate was 0.075%. A high percentage of stillbirth (5.13%) from heifers the first fertilization event, and there was a high rate of abortions in older cows (11 calving, 1.76%). An analysis of 62,534 records of bulls with the rs475678587C>T variant of UBE3B gene (AH1 haplotype) (n = 69, of which 51 bulls were free from and 18 were carriers of AH1) for the period 2002 to 2018 showed that the proportion of female offspring of AH1-C bulls completing at least one full period of lactation was about one-third of the total livestock. An analysis of the results of mating with AH-F and AH-C bulls for the broodstock as a whole (without taking into account the female genotype) did not reveal significant differences in the number of stillbirths and abortions. Linear regression analysis showed that the status of AH1 bulls did not significantly affect stillbirth rates. An analysis of the results of calving and the reproductive ability of the offspring of AH-C bulls showed an increase in the frequency of stillbirths, confirmed by the results of linear regression analysis, which revealed a significant effect of bull fathers carrying AH1 on the proportion of stillbirths experienced by their daughters. Our studies confirm that the rs475678587C>T variant of the UBE3B gene (haplotype AH1) has a significant effect on the rate of stillbirth of a cow or heifer if their father is a carrier of this genetic defect.

Keywords: genetic defect; fertility; abortion; dairy cattle

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ВАРИАНТ RS475678587C>T ГЕНА UBE3B (ГАПЛОТИП АН1) У АЙРШИРСКОГО СКОТА, ПОВЫШАЮЩИЙ РИСК МЕРТВОРОЖДЕНИЯ У ДОЧЕРЕЙ БЫКОВ-НОСИТЕЛЕЙ

О.В. Тулинова, Е.Н. Васильева, Е.А. Романова, Ю.С. Щербаков, М.В. Позовникова

Целью нашего исследования был анализ динамики воспроизводства поголовья айрширского скота в России, а также оценка уровня влияния гаплотипа АН1 на частоту мертворождений. Для общей оценки рождаемости мы использовали 390769 записей 67 молочных стад в России с 2003 по 2018 г. На фоне стабильного прироста поголовья средняя мертворождаемость составила 3,33%, абортов – 0,075%. Выявлен высокий процент мертворождений (5,13%) у телок при первом оплодотворении, а также высокий уровень абортов у коров старшего возраста (11 отелов, 1,76%). Анализ 62534 записей у быков с вариантом rs475678587C>T гена UBE3B (гаплотип АН1) (n = 69, из них 51 бык был свободен от АН1 и 18 были носителями) за период с 2002 по 2018 г. показал, что доля потомства самок быков АН1-C, завершивших хотя бы один полный период лактации, составляла около одной трети всего поголовья. Анализ результатов спаривания самок с быками АН-F и АН-C по маточному стаду в целом (без учета генотипа самок) не выявил существенных различий по количеству мертворождений и абортов. Линейный регрессионный анализ показал, что статус быков АН1 существенно не влиял на уровень мертворождаемости. Анализ результатов отелов и воспроизводительной способности потомства быков АН-C показал увеличение частоты мертворождений, подтвержденное результатами линейного регрессионного анализа, выявившего достоверное влияние отцов-быков-носителей АН1 на долю мертворождений, у их дочерей. Наши исследования подтверждают, что вариант rs475678587C>T гена UBE3B (гаплотип АН1) оказывает существенное влияние на частоту мертворождения у коровы или телки, если их отец является носителем этого генетического дефекта.

Ключевые слова: генетический дефект; фертильность; аборт; молочный скот
Introduction

Herd reproduction largely determines the economic well-being of a farm. Each successful calving is associated with healthy offspring and the initiation of lactation. According to [18], a decrease in herd fertility may be largely due to the presence of recessive lethal mutations. Past research shows that, among other causes, about 5% of cases of perinatal mortality on average are due to genetic defects [20]. With the introduction of mass DNA genotyping of animals using high-density chips in the practice of animal husbandry, information has become available on lethal genetic defects that cause fetal developmental disorders during embryogenesis. These mutations were identified as fertility haplotypes, although random mutations were later identified for many of them [11, 26]. Previously, only milk traits were included in genomic assessments. Today such assessments also examine reproductive qualities, including the presence of recessive alleles that cause fatal effects when present in a homozygous state. This approach allows not only significant improvements in herd productivity but also reduced costs and losses caused by abortion and stillbirth [12]. For Ayrshire cattle, only two responsible haplotypes have been identified to date: Ayrshire haplotype 1 (AH1; OMIA 001934) and Ayrshire haplotype 2 (AH2; OMIA 002134). Haplotype AH2 is associated with early embryonic death. The cause of AH2 is disruption of the RPAP2 gene on the BTA3 chromosome, which plays a key role in the early stages of embryogenesis; specifically, it is involved in RNA synthesis [6]. This haplotype was reported for the first time in 2017 by Null et al., and according to the data they provided, the frequency of AH2 in US Ayrshires was 18.9%. Information on the AH1 haplotype became available in 2014 when whole-genome sequencing was used to map it to a region on BTA17 in the range of 65.9 to 66.2 Mbp, associated with impaired fertility in cows [9]. Later, it was found that this haplotype was associated with SNP rs475678587, Chr17: 65,921,497 C >T in UMD3.1 in exon 23 of the UBE3B gene (coding for ubiquitin protein ligase E3B). This mutation leads to exon skipping in a reading frame and, as a consequence, partial truncation of the HECT domain in the UBE3B protein. UBE3B mutation causes the clinically recognizable PIRM syndrome (ptosis, retarded growth, intellectual disability, and mortality) in calves. Calves homozygous for the AH1 haplotype die late in
gestation or in the early postnatal period [28]. Studies show a high incidence of AH1 in Ayrshire cattle herds in different countries, such as Finland (17.1%) [28] and the USA (22.2%) [21].

In our previous studies, we showed that for the Russian population of Ayrshire cattle, the AH1 haplotype is a serious problem due to the high percentage of carriers among bulls (16.66%) and its significant negative impact on the number of stillbirths, which increases when the daughters of AH1-C bulls are mated with AH1-C bulls [22]. However, the issue of predicting stillbirth when using a bull-producer, in general, and when assessing the results of calving daughters of bulls has not been studied.

The aims of our study were as follows: (i) to analyze the dynamics of calf fertility in Ayrshire cattle in Russia, (ii) to assess the level of influence of the AH1 haplotype on the rate of stillbirth, (iii) to prospectively study how the AH1 status of a bull affects the calving results following mating with a female, and (iv) to conduct a prospective study of the effect of AH1 producers status on the calving success of their daughters.

Materials and methods

Animal genotypes

The genotypes of the used bulls had been characterized in our previous study. Briefly, 69 Ayrshire bulls used in the Russian artificial insemination system were tested, of which 18 animals were heterozygous carriers of AH1 (AH1-C) and 51 were noncarriers (AH1-F). Genotyping for AH1 carriage was performed using the Sanger sequencing method (rs475678587) [22]. In short, DNA was obtained from bovine semen using standard phenol–chloroform extraction. For amplification, primers F: 5’-AGCAGCGGTGTCATTCTGTGAG-3’ and R: 5’-CACTGTTGACCCCATTTCCG-3’ (Eurogen CJSC, Moscow, Russia) were used, and an amplified fragment of 250 bp was obtained. Preliminary amplification was performed using a Thermal Cycler T100 (Bio-Rad, USA). Sequencing was performed on an Applied Biosystems 3500 Genetic Analyzer using a commercial BigDye® Terminator v3.1 Sequencing Standard Kit (Applied Biosystems) according to the accompanying instructions. Alignment and analysis of DNA fragments were performed using Mega-6 software [19].

Dynamic analysis

A total of 390,769 records of 67 dairy herds in Russia from 2003 to 2018 were included in the dataset for the dynamic analysis. The number of stillbirths and abortions of cows and heifers were analyzed according to year and lactation status. In all 62,534 records from 2002 to 2018 were reviewed to analyze the number of daughters produced from fathers with AH1 status that had completed
at least one lactation. Data from the SELEX database (RC Plinor LLC) were used. The primary data processing was carried out in the Selection and genetic statistics (SGS-VNIIGRZH) program [24].

**Analysis of the influence of bull haplotype on the fertility of cows and heifers according to AH1 status**

Using data on bulls of the Ayrshire breed (n = 69; 133,119 records, including 36,250 records for 18 AH1-C bulls and 96,858 records for 51 AH1-F bulls), the influence of a bull inseminator on the fertilization rate and calving results of cows and heifers was studied. The analysis included the frequency of insemination to the first calving and the results of calving in terms of the numbers of live calves, stillbirths, and abortions. For bulls with a score for offspring (n = 46; 4852 records, including 1683 records for 11 AH1-C bulls and 3169 records for 35 AH1-F bulls), the reproductive indicators of their daughters were analyzed, such as age at first calving, frequency of insemination to first calving, frequency of insemination after first calving, service period, and results of calving by the numbers of live calves, stillbirths, and abortions.

**Statistics**

Statistical analysis was carried out using Statistica data analysis software v13 (Dell Inc. 2016, software.dell.com). ANOVA was performed at a significance level of p < 0.05 to compare mean values in groups. Linear regression, reflecting the influence of sire bulls and bull-inseminators on the viability of offspring, was calculated in the R statistical package v.4.0.2 environment. The linear regression model was built according to Eq. (1):

\[ Y = (a + b) \cdot X \]  

where Y is live and stillborn offspring and X is AH1 carrier and noncarrier bulls. The models were visualized in R programming language using the linear model (LM) function implemented in R.

Correlation analysis was performed using Spearman’s rank correlation coefficient.

**Results and discussion**

**Dynamic analysis of the results of calving for Ayrshire cows and heifers**

A total of 390,769 records of calving cows and heifers from 67 dairy herds in Russia from 2003 to 2018 were analyzed, the relative proportion of which grew from 2.50% in 2003 to 8.52% in 2018, which indicates a stable livestock increase in the analyzed population. Over their productive life, cows had an average of 2.7 lactations, including 1.02 offspring (397,253 heads) or 0.98 live calves (384,037 heads) were obtained. The numbers of stillbirths and abortions recorded in the given period are shown in Figure 1a.
A total of 2925 abortions were registered, which corresponds to 0.75% of all recorded calving events, and stillbirths occurred in 13,216 cases (3.33%). A high stillbirth rate of 4.18% was recorded in 2003. Stillbirths increased from 3.38 to 3.97% in the period 2006 to 2010. Minimum values were recorded in 2005 (2.83%), 2015 (2.81%), 2017 (2.78%), and 2018 (2.82%). The percentage of abortions varied within the range 0.55-0.88% over the entire period, except for 2018, when it was 0.94%.

The analysis shows that cows calving for first time were characterized by a high percentage of stillbirths (5.13%) and a low percentage of abortions (0.41%) (Figure 1b). Significant increases in abortions were observed at the 4th calving (1.05%), 9th calving (1.31%), and 11th calving (1.76%). The stillbirth rate did not exceed 2.71% from the 2nd lactation, and was 1.89% at the 10th lactation. Figure 1c shows the dynamics of the number of heifer descendants included in the analysis of bulls by birth year, showing stable livestock growth during the analyzed period. The percentage of descendants of AH1-F bulls was significantly higher, varying between 67.62 and 95.29%, with the exception of 2004, when they accounted for only 57.33%. The number of descendants of AH1-C bulls also steadily increased, and the maximum values were observed in 2004, 2016, and 2017 (42.67, 31.67, and 32.3%, respectively).

**Analysis of calving results and reproductive qualities of cows and heifers when mated with AH1-F and AH1-C bulls**

There was a reliably high rate of live calves in the group of AH1-F insemination bulls, which may be related to the sample size (Table 1); there were no significant differences in the rate of stillbirths. There was a higher negative correlation between the rate of live calves and stillbirths in the group of AH1-C insemination bulls: \( r = -0.949 \) (\( p < 0.05 \)). The correlation coefficient was determined at the level of \( r = -0.813 \) (\( p < 0.05 \)) for AH1-F insemination bulls.
### Table 1.

Calving results and reproductive qualities of cows and heifers, taking into account the AH1 status of the producer bull

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bulls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AH1-F (n = 51)</td>
<td>AH1-C (n = 18)</td>
</tr>
<tr>
<td>Records</td>
<td>96869</td>
<td>36250</td>
</tr>
<tr>
<td>Number of inseminations to 1st calving (M ± SEM)</td>
<td>1.93 ± 0.004</td>
<td>1.91 ± 0.006</td>
</tr>
<tr>
<td>Number of calves (M ± SEM)</td>
<td>0.980 ± 0.002</td>
<td>0.978 ± 0.005</td>
</tr>
<tr>
<td>Abortion rate (M ± SEM)</td>
<td>0.0063 ± 0.0006</td>
<td>0.0057 ± 0.0009</td>
</tr>
<tr>
<td>Stillbirth rate (M ± SEM)</td>
<td>0.036 ± 0.002</td>
<td>0.038 ± 0.005</td>
</tr>
</tbody>
</table>

AH1-C, AH1 fertility haplotype carrier; AH1-F, AH1 noncarrier; M, mean; SEM, standard error of the mean.

**Analysis of calving results and reproductive qualities of cows and heifers: daughters of AH1-F and AH1-C bulls**

The stillbirth rate (p < 0.001) in daughters produced from AH-C bulls increased significantly relative to the decreased number of live calves (Table 2). A significant negative relationship was revealed between these indicators. The correlation coefficient was r = −0.984 (p < 0.05). A negative correlation was also observed in the group of daughters produced from AH1-F bulls, although values were lower (r = −0.719, p < 0.05). A comparison of analyzed animal groups showed that daughters produced from AH1-C bulls differed by a shorter service period (p < 0.001) and fewer sperm doses for successful insemination after the first calving (p < 0.002).

### Table 2.

Calving results and reproductive qualities of cows and heifers taking into account the AH1 status of father bulls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bulls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AH1-F (n = 35)</td>
<td>AH1-C (n = 11)</td>
</tr>
<tr>
<td>Records</td>
<td>3169</td>
<td>1683</td>
</tr>
<tr>
<td>Age at 1st calving, months (M ± SEM)</td>
<td>26.52 ± 0.05</td>
<td>26.64 ± 0.07</td>
</tr>
<tr>
<td>Service period, days (M ± SEM)</td>
<td>136.49 ± 1.48</td>
<td>128.27 ± 2.03</td>
</tr>
<tr>
<td>Number of inseminations to 1st calving (M ± SEM)</td>
<td>2.15 ± 0.02</td>
<td>2.12 ± 0.03</td>
</tr>
<tr>
<td>Number of inseminations after 1st calving (M ± SEM)</td>
<td>1.806 ± 0.021</td>
<td>1.696 ± 0.029</td>
</tr>
<tr>
<td>Number of calves (M ± SEM)</td>
<td>0.988 ± 0.003</td>
<td>0.960 ± 0.005</td>
</tr>
<tr>
<td>Abortion rate (M ± SEM)</td>
<td>0.003 ± 0.001</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>Stillbirth rate (M ± SEM)</td>
<td>0.024 ± 0.003</td>
<td>0.047 ± 0.004</td>
</tr>
</tbody>
</table>

AH1-C, AH1 fertility haplotype carrier; AH1-F, AH1 noncarrier; M, mean; SEM, standard error of the mean.
**Linear regression analysis**

The data in the linear regression plot (Figure 2) did not support the effect of the AH1 genotype of bull inseminators in terms of the probability of stillborn offspring (multiple R-squared = 2.287e-05, adjusted R-squared = 1.536e-05, F-statistic coefficient = 3.047, p = 0.0809).

![Linear regression plots](image)

**Fig. 2.** Linear regression plots for effect of AH1 haplotype carrier and noncarrier bovine inseminators, based on prediction model for stillbirth in the case of mating bulls. Residuals vs. fitted: shows unaccounted for curvilinear trends. Normal Q-Q: shows normal distribution of residuals. Scale-location displays hetero/homoscedasticity and detects outliers. y-axis represents square roots of error modulus. Residuals vs. leverage: x-axis is Cook’s distance, y-axis is standardized size of outliers.

The bull father effect, taking into account the AH1 genotype, was modeled to assess the effect on stillbirth in female offspring. The level of reliability of the model was p = 2.668e-05 (with R-squared = 0.003642, adjusted R-squared = 0.003436, F-statistic coefficient = 17.67; Figure 3). The obtained data indicate that the presence of the AH1 mutant allele in the bull genotype can significantly increase the risk of stillbirth for the daughters of these bulls.
Fig. 3. Linear regression plot for effect of AH1 haplotype carrier and noncarrier bull fathers, based on prediction model for stillbirth in female offspring. Residuals vs. fitted: shows unaccounted for curvilinear trends. Normal Q-Q: shows normal distribution of residuals. Scale-location displays hetero/homoscedasticity and detects outliers. y-axis represents square roots of error modulus. Residuals vs. leverage: x-axis is Cook’s distance, y-axis is standardized size of outliers.

**Discussion**

*Dynamic analysis of the results of calving Ayrshire cows and heifers*

Our data show that the stillbirth rate in the analyzed Ayrshire cattle population decreased from 4.18% in 2003 to 2.82% in 2018 with an increase in the relative number of calving events. The opposite trend was found for the abortion rate, which increased from 0.55 to 0.94% from 2003 to 2018. The average stillbirth rate in the analyzed population of Ayrshire cattle in Russia was 3.33%, which is lower than in populations in other breeds and countries, and the constant rate over time is in contrast with other reports. Donald (1963) reported a stillbirth rate of 4.7% for Ayrshire cows. In 2007, Cole et al. reported that the proportion of stillbirths in Ayrshire herds was 5.5%. An analysis of mortality in 82 Ayrshire herds in Finland showed that the mortality risk for calves less than 7 days old averaged 5.2 ± 2.3% [23]. Winder et al. reported data from the Canadian National Dairy Study in 2018, showing that the average stillbirth rate in dairy herds was 4.9%.
Studies have shown strong genetic correlations between stillbirth rates and calving difficulty [15], which is also more common in first-calving cows [3]. Juozaitiene et al. (2018) reported that the stillbirth rate in primiparous heifers was 5.367%, which was 0.5-2% higher than in multiparous cows (p < 0.0001); according to the authors, this may be more associated with the difficulty of calving. Our study shows a high percentage of stillborn calves for primiparous cows, 5.13%, which decreased to 2.43% after the second calving. The incidence of stillbirth at first and second calving was assessed in Finnish Ayrshires in studies by Hietala et al. (2014), taking into account complications during calving. In the case of easy first calving, 3.3% of stillborn calves were recorded and the rate was 1.8% at second calving. With complications occurring in calving, the rate increased to 18.8% for first calving and 15.2% for second calving.

Abortion is a significant negative factor. Our data show that the average abortion rate was 0.75% from 2003 to 2018, with a lower percentage of 0.41% at first calving, which is significantly lower in comparison with Finnish Ayrshires, among which the percentage of abortions was 1.3% at first calving and 1.7% at second calving [16].

An analysis of the number of descendants of daughters produced from AH1 carrier and noncarrier bulls showed a stable increase in livestock in the entire sample and in bull groups. It is alarming that in 2018, 28.6% of daughters were produced from AH1-C bulls, which comprise about one-third of the total analyzed livestock. Although the heifer genotypes have not been determined, we assume that there is a 50% chance that heifers will inherit the AH1 haplotype, which may adversely affect herd fertility and increase stillbirths. In 2017, CDN published data on trends in the average probability of AH1 carriage among Canadian Ayrshire cows. The analysis included data on heifers born from 1980 to 2016. The frequency of AH1 fluctuated between 17 and 30% over the years [4]. According to the latest data, among cows born in 2020, 17.4% were carriers [5]. This information is important and should be available to farmers, as it makes it possible to predict the numbers of carriers and noncarriers of AH1 in herds and in the population as a whole.

**Analysis of calving results and reproductive qualities of cows and heifers when mating with AH1-F and AH1-C bulls**

The evaluation of the results of mating inseminating bulls, according to AH1 genotype, with heifers whose pedigree and genotype were not considered did not reveal significant differences in the numbers of stillbirths and abortions, or in the rate of frequency of insemination by first calving. Our early studies showed that bull inseminators carrying the AH1 haplotype can have a negative impact.
on the stillbirth rate only when mating with cows and heifers that are daughters of AH1-C bulls [22]. Therefore, the AH1 status of inseminating bulls should not be ignored, since if it is impossible to genotype females, it may be effective to analyze pedigrees, taking into account the status of the maternal father and grandfather to calculate the probability of homozygous births in the herd [26].

**Analysis of calving results and reproductive qualities of cows and heifers that are daughters of AH1-F and AH1-C bulls**

An analysis of the calving results of cows and heifers taking into account the AH1 status of the father bull showed that significantly more calves were produced from daughters of AH1-F bulls (p < 0.000). Apparently, this is mainly determined by the size of the sample, but it may also be a favorable factor determining an increase in the number of animals free from AH1.

In our study, the daughters of AH-C bulls had a shorter service period (p < 0.001), and fewer sperm doses were spent for their insemination after the first calving (p < 0.002), which indicates the females had good fertility. At the same time, we observed a significantly high stillbirth rate (p < 0.000). It can be assumed that in this case the high average stillbirth rate is associated with a high probability of females inheriting AH1, and their mating with AH1-C bulls therefore has a negative effect on the viability of the offspring. In earlier studies, to assess the effect of the AH1 haplotype on the birth of nonviable offspring, the results of mating of bulls and females were analyzed only taking into account the AH1 status of bulls and father-bulls. A significant increase in stillbirths was observed when mating AH1 bulls with daughters of AH1 bulls [14,22,25]. This is alarming, since all available records were taken into account when registering stillbirths, without a detailed analysis of the causes of each, although factors such as trauma and/or infectious disease in the mother, malnutrition, and energy imbalance can also be causes of stillbirth [1]. In our work, we show, for the first time, that the risk of having a dead calf is increased in heifers and cows that are daughters of AH1-C bulls. It can be concluded that these data will undoubtedly be useful in the preparation of mating programs, and the conclusions are consistent with the those of other studies [2].

**Linear regression analysis**

This assumption is supported by linear regression analysis, which revealed a significant influence of AH1-C sire bulls on the birth of nonviable offspring in their daughters. In general, predicting the influence of an animal genotype on its further reproductive capacity is important to create systems for managing recessive mutations in herds. For example, the approach developed by [8] made it possible to reduce the frequency of not only recessive but also low-frequency
mutations in the population. The genomics-based model includes limiting inbreeding and accounting for economic losses based on the Mendelian sample. The model presented by Upperman et al. (2019) made it possible to exert control over the relationship of parents and descendants by conducting simultaneous selection and selection of pairs in order to avoid the birth of homozygous individuals. Management of recessive mutations in a herd is always a choice between excluding bulls from reproduction and eliminating negative alleles in the new generation [8]. However, the elimination of bulls carrying genetic defects is not always an effective method in practice, as it can lead to a sharp reduction of sires in the population and decreased genetic diversity. A more efficient method today is to create models that estimate the cost of a defect and determine the economic value of other traits based on the breeding value index. Genotyping of females is also desirable to better select parental pairs [13].

**Conclusion**

Dynamic analysis showed an increase in the number of Ayrshire cattle in the Russian Federation with an average stillbirth rate of 3.33% and abortion rate of 0.75%. Most of the analyzed population comprised AH1 noncarrier bulls, which made up more than 70% in 2018. There was no negative effect of bulls taking into account the status of AH1 on the stillbirth rate. While daughters produced from AH1-C bulls showed a significant increase in the stillbirth rate, this group was characterized by a shorter service period and a lower frequency of inseminations after the first calving. Linear regression analysis revealed a significant effect of sire bulls carrying AH1 on the rate of stillbirth in their daughters, which indicates the need to select parental pairs taking into account the AH1 status and pedigree of animals.

**Ethical statement.** Ethics approval was not required in the study as no animal experiments were performed.

**Conflict of interest information.** The authors declare no conflict of interest.

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**References / Список литературы**


**AUTHOR CONTRIBUTIONS**

Olga V. Tulinova: conceptualization, general direction of the scientific direction.
Ekaterina N. Vasilyeva: preparation of the text of the article.
Elena A. Romanova: preparation of the text of the article, editing the manuscript of the article.
Yuriy S. Shcherbakov: interpretation of results, statistical analysis of data.
Marina V. Pozovnikova: general management of the direction of the study, interpretation of the results, statistical analysis of data, preparation of the text of the article, responsibility of the author for all aspects of the work.

**ВКЛАД АВТОРОВ**

Тулинова О.В.: концепция исследования, общее руководство направлением исследования.

Васильева Е.Н.: написание текста статьи.

Романова Е.А.: написание текста статьи, редактирование рукописи статьи.

Щербаков Ю.С.: интерпретация результатов, статистический анализ данных.

Позовникова М.В.: общее руководство направлением исследования, интерпретация результатов, статистический анализ данных, подготовка текста статьи, ответственность автора за все аспекты работы.
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