

ANIMAL BREEDING, SELECTION, GENETICS, AND BIOTECHNOLOGY

РАЗВЕДЕНИЕ, СЕЛЕКЦИЯ, ГЕНЕТИКА И БИОТЕХНОЛОГИЯ ЖИВОТНЫХ



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Finding Associations of Single Nucleotide Polymorphisms with Exterior Traits (Pelvic Parameters) in Holstein CowsMaksim V. Bytov ✉, Vladlena D. Zubareva , Alexander S. Krasnoperov ,Natalia A. Bezborodova , Marina A. Pechenkina , Olga V. Sokolova

Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russian Federation

✉ bytovmaks@mail.ru**Abstract**

Introduction. Exterior traits of a cow can have crucial effect on development of pathological conditions, for example, sacrum anatomy features can be related to the risk of dystocia (pathological or difficult parturition). In dairy farming, pelvic parameters are one of the key factors enabling control of easy calving. However, modern breeding focuses on selection of the herd individuals with the highest milk yield and long-term economic productivity, whereas the exterior traits, unfortunately, are taken into account to a lesser extent. The aim of the present research is to identify associations of single-nucleotide polymorphisms with cow pelvic exterior parameters that are important prerequisites of susceptibility to easy or difficult calving. As a result, it will be possible to determine the genetic markers to be included into the selection-breeding programs.

Materials and Methods. The study was conducted at Ural Research Veterinary Institute — a structural division of Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences in the period from 2023 to 2025. The exterior trait inheritance patterns were studied in Holstein cows (n=155) across three pelvic dimensions: sacrum height, hip width, and ischial tuberosity width. Association tests were conducted using SNPAssoc R package.




Results. The mean values and standard deviations for pelvic height at sacrum in cows were found to be 161.7±5.3 cm; for width at hips — 41.1±3.9 cm; for width at ischial tuberosities — 22.4±2.1 cm. For each individual, genotyping with 13 polymorphisms was performed. Rs109452259 polymorphism in C* allele was revealed to have negative effect on hip width in Holstein cows. Rs134055603 and rs43038601 polymorphisms in G/G and C/A genotypes respectively, were established to have putative epistatic interaction with positive effect on the sacrum height. Also, rs134055603 and rs137396952 polymorphisms in C/A and C/C genotypes respectively were found to have possibly synergistic effect.

Discussion and Conclusion. The data obtained in the frame of the study are reliable for the observed-level statistical interaction. Potential limitations of the study include possible influence of the unaccounted confounding factors, such as variability of animals within the studied groups by age, live body weight, lactation number, etc. For confirmation of the biological mechanisms, further functional studies are required.

Keywords: cows, Holstein breed, exterior traits, sacrum height, hip width, ischial tuberosity width, polymorphisms, genetic associations, selection-breeding

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Поиск ассоциаций однонуклеотидных полиморфизмов с показателями экстерьера (тазовые параметры) коров голштинской породы**М.В. Бытов**  , **В.Д. Зубарева** , **А.С. Краснощёров** , **Н.А. Безбородова** , **М.А. Печёнкина** ,
О.В. СоколоваУральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук,
г. Екатеринбург, Российская Федерация bytovmaks@mail.ru**Аннотация**

Введение. Экстерьерные параметры коровы могут иметь решающее значение в развитии патологического состояния, например, особенности строения крестца могут быть связаны с риском дистоции (патологические или трудные роды). Параметры таза являются одним из ключевых факторов управления легкостью отела в молочном скотоводстве, однако современная селекция направлена на закрепление в стаде особей с наибольшей молочной продуктивностью и длительным хозяйственным использованием, и экстерьерные параметры, к сожалению, учитываются в меньшей степени. Цель исследования — выявить ассоциации однонуклеотидных полиморфизмов с экстерьерными показателями таза коров, играющими важную роль в предрасположенности к легкому или трудному отелу, что позволит определить генетические маркеры для использования в селекционных программах.

Материалы и методы. Исследование проведено в Уральском НИВИ — структурном подразделении ФГБНУ УрФАНИЦ УрО РАН в период с 2023 по 2025 гг. Исследованы модели наследования экстерьерных параметров по трем проекциям таза коров голштинской породы (n=155): высота в крестце, ширина в маклоках, ширина в седалищных буграх. Ассоциативные тесты проведены с использованием SNPAssoc для программной среды R.

Результаты исследования. Установлено, что средние значения и стандартные отклонения для высоты таза коров в крестце равны 161,7±5,3 см; для ширины в маклоках — 41,1±3,9 см; для ширины в седалищных буграх — 22,4±2,1 см. Проведено генотипирование каждой особи по 13 полиморфизмам. Для коров голштинской породы по rs109452259 выявлен отрицательный эффект аллеля C* на ширину в маклоках. Обнаружено предположительно эпистатическое взаимодействие для rs134055603 и rs43038601 для генотипов G/G и C/A, соответственно, с положительным эффектом на высоту в крестце. Также обнаружено эпистатическое взаимодействие для rs134055603 и rs137396952, вероятно, с синергическим характером для генотипа C/A и C/C соответственно.

Обсуждение и заключение. Полученные в ходе исследования данные статистически достоверны для наблюдаемого взаимодействия. К потенциальным ограничениям исследования следует отнести возможное влияние неучтенных смешивающих факторов, таких как вариабельность внутри исследуемых групп по возрасту животных, живой массе, номеру лактации и т. д. Подтверждение биологических механизмов требует дополнительных функциональных исследований.

Ключевые слова: коровы, голштинская порода, экстерьерные показатели, высота в крестце, ширина в маклоках, ширина в седалищных буграх, полиморфизмы, генетические ассоциации, селекция

Финансирование. Работа выполнена в рамках Государственного задания Министерства науки и высшего образования Российской Федерации по теме № 0532-2025-0003 «Разработка высокоточных методов идентификации генетических детерминант устойчивости к заболеваниям для использования в селекции крупного рогатого скота».

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Introduction. In modern animal husbandry, the exterior of cattle is an important production indicator directly affecting health, productivity, and reproductive function [1]. Body constitution indices are taken into account in dairy cow breeding among the primal indicators to assess the non-productive traits [2]. Gibson et al. believe that height of a calf is related to the lactation efficiency of an adult cow: taller calves will produce more milk in the future through several

lactations compared to the shorter calves, live weight considered [3]. The results of the study conducted by Schmidtman et al. show that larger animals with visibly protruding bones and a low BCS (Body Condition Score) are more susceptible to metabolic disorders [4].

Exterior traits can be decisive for the development of pathological conditions in animals. For example, sacrum anatomy features can be related to the risk of dystocia

(pathological or difficult parturition) [5]. Significantly higher incidence of dystocia has been found in cows with the sacrum size both narrower and, on the contrary, wider than optimal for calving [6]. The influence of a breed on the incidence of dystocia is explained by the differences in a relative body weight at birth, pelvis anatomy, and significant variations in dimensions of pelvis in some breeds [5]. Due to modern breeding focusing on selection of the herd specimens with the highest milk yield and long-term economic productivity, dystocia remains a serious problem with incidence rate estimated at 10 to 50% [7].

The aim of the study is to identify associations of single-nucleotide polymorphisms with pelvic exterior parameters in Holstein cows that are important prerequisites for forming susceptibility to easy or difficult calving. The data obtained will make it possible to determine the genetic markers to be included into the herd improvement breeding programs.

Materials and Methods. The study was carried out at the Department of Genomic Research and Animal Breeding of Ural Research Veterinary Institute, structural division of Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences, in the period from 2023 to 2025. PCR tests of DNA samples from Holstein cows ($n=155$) from two cattle breeding agricultural enterprises (AEs) (AE No. 1, $n=70$; AE No. 2, $n=85$) were performed. The research sample included animals with one or more lactations. The research was held in compliance with the ethical standards, the studies (e.g., blood sampling from the caudal veins of animals) were approved by the Bioethics Committee of Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences (Protocol No. 136 of October 13, 2025).

Exterior traits of the animals were assessed across three dimensions: sacrum height, hip width, and ischial tuberosity width. Statistical data for a phenotype — mean values and standard deviations — were calculated using STATISTICA 12 software (StatSoft Inc, USA).

13 Single Nucleotide Polymorphisms (SNPs) were genotyped across entire sample of cows. To perform association tests, the following polymorphic markers were studied: rs134055603, rs137396952, rs109452259, rs110347054, rs110352004, rs133674837, rs109529386, rs109279094, rs109611915, rs134668940, rs41567027, rs43038601, rs41255693, which had previously shown significant associations with a number of physiologically valuable parameters in cattle during GWAS studies:

- productive longevity [8];
- easy calving [9, 10];
- body weight [11];
- carcass length after cutting [12];
- and other parameters of productivity [10, 13].

The article presents both authors' own findings based on TaqMan genotyping technology and earlier described test systems (Table 1).

Based on the genetic data obtained, the relationship of each polymorphic locus with a phenotype was assessed in separate. Five patterns of trait inheritance were assessed using the SNPStats web tool (Institut Català d'Oncologia, Spain) [19] and the SNPAssoc package [20] in R programming environment (Autonomous University of Barcelona, Spain): codominant, dominant, recessive, overdominant, and log-additive; the effect size was interpreted as the odds ratio (the differences in the values of the exterior traits) with a 95% confidence interval compared to alternative allele homozygote.

To conduct association studies using the SNPAssoc package in the R programming environment, the WGassociation function was used. To test for the presence of “gene-gene” interactions, the interactionPval (Phen, data=data, model='co') function was used. Association tests were performed across three pelvic dimensions: sacrum height, hip width, and ischial tuberosity width; the presence of “gene-gene” interactions was also assessed for each of the exterior traits. For a more detailed study of allele combinations contributing to the formation of a trait, taking into account “gene-gene” interactions, a linear model was created: $\text{lm}(\text{"Phen ~"}, \text{"SNP1"}, \text{"**"}, \text{"SNP2"}, \text{data=data})$. With regard to epistatic interactions, this model envisaged sorting through each combination of genotypes and determining mean value of each of these combinations for a phenotype. A post-hoc Bonferroni correction was performed for each analysis

Results. The mean values and standard deviations for sacrum height in Holstein cows were determined to be 161.7 ± 5.3 cm; for hip width — 41.1 ± 3.9 cm; and for ischial tuberosity width — 22.4 ± 2.1 cm.

Larger values of pelvic width proved to ensure sufficient space for successful development of a fetus and easy calving, and necessary space between hindlimbs to ensure a more voluminous cup-shaped udder [21]. Thus, higher values of each of the three exterior traits are considered a favourable phenotype.

Significant effects of rs109452259 SNP on hip width in Holstein cows were acknowledged in codominant, dominant, and log-additive inheritance patterns. The lowest AIC value for rs109452259 was found in the dominant inheritance pattern, with the C/A and A/A genotypes having 2.17 cm difference from C/C; $p\text{-value}=0.0006$.

When studying a phenotype inheritance mechanism, an interesting feature was revealed, i.e. the presence of “gene-gene” interactions among polymorphisms. In the present study, the epistatic interactions for the pairs of rs134055603–rs43038601 and rs134055603–rs137396952 polymorphisms were identified according to the codominant inheritance pattern (Fig. 1). The significance level for these pairs was corrected to $\alpha < 0.00032$.

Oligonucleotides used in the present study for genotyping in cattle

SNP, localization and genomic context	Oligonucleotides	Amplicon, bp	Source
rs133674837 6:21766662 <i>BDH2</i>	F, AAAGAAGGTGCCAAAGT	91	This study
	R, TCAAGCTATGGAGGTGCT		
	P1, [R6G]ATCAATGACTCCAAACT[BHQ1]		
	P2, [ROX]ATATCAATGAATCCAAACT[BHQ2]		
rs41567027 6:43657946 <i>PPARGC1A</i>	F, CACTAGAAAGCACACTTCA	197	This study
	R, ATACTTGGCTGTGTGGAA		
	P1, [R6G]AGAAGCCGCAAGGTCA[BHQ1]		
	P2, [ROX]CAGAAGCTGCAAGGTCA[BHQ2]		
rs109279094 6:45175137 <i>PPARGC1A</i>	F, ACTAAACCTCTCTGTCTT	338	This study
	R, GATTTGTGTCAGTCTCT		
	P1, [R6G]TCCCCAGACTCATAA[BHQ1]		
	P2, [ROX]CTCCCCTAGACTCATAAC[BHQ2]		
rs109611915 6:88739941 <i>GC – NPFFR2</i>	F, CCTTGTAATGCAGAATCCAC	132	This study
	R, GAACCAACGTTGACCTGAT		
	P1, [R6G]TCATTTTGAGATAACAGAAC[BHQ1]		
	P2, [ROX]TCATTTTGAAGATAACAGAACTA[BHQ2]		
rs110347054 6:88751491 <i>GC – NPFFR2</i>	F, GGAGCTGGGATTGATGCCTAC	226	[14]
	R, AAGAAAATCA+CA+CTTCAAAAGGATA		
	P1, [ROX]CCTACTCCCTC+C+A+CTGGGTG[BHQ2]		
	P2, [Cy5]CCTACTCCCTCC+G+CTGGGTG[BHQ2]		
rs109452259 6:88800322 <i>GC – NPFFR2</i>	F, GCAAAAACACAATATGCTGGAT	415	[16, 15]
	R, AGGTCAAACAACATAACAGTGG		
	P1, [ROX]CTTGTC+A+A+CTT+C+CA[BHQ2]		
	P2, [FAM]CTTGTC+A+C+CTTCCA[BHQ1]		
rs137396952 6:88817457 <i>GC – NPFFR2</i>	F, ATGCAGCAGAAACAAGGGTAAA	225	[17]
	R, GTACAGCCACTGTGCAACAAC		
	P1, [HEX]GA+TT+CAGCATG+G+T+G+TCAG[BHQ2]		
	P2, [Cy5]GATT+CAGCATG+G+C+G+TCAG[BHQ3]		
rs134055603 6:88832335 <i>GC – NPFFR2</i>	F, GACAAGGCTTTTGTAGGTGAAA	316	[17]
	R, CAAAGCAACCACACAATGTTG		
	P1, [HEX]CAT+TT+TCT+T+A+GA+CT+T+CTG[BHQ1]		
	P2, [Cy5]CATTTTCT+T+G+GA+CT+T+CTG[BHQ3]		
rs134668940 6:88838658 <i>GC – NPFFR2</i>	F, GGCAGAGAACTTGACT	276	This study
	R, AGTATCTTG+GCCTCTT		
	P1, [R6G]AGAATAGCACATGGCACA[BHQ1]		
	P2, [ROX]TAGAATAGCAAATGGCACATA[BHQ2]		
rs110352004 6:88948552 <i>GC – NPFFR2</i>	F, GTAGGGATT+GAT+GC+CCTTGAA	232	[14]
	R, TACAATA+CA+C+CATAT+CTTTTCATCC		
	P1, [HEX]AA+TA+C+GTAC+AA+CACT+CT+T[BHQ1]		
	P2, [ROX]TA+C+GTAC+GA+CACTCTGT[BHQ2]		
rs109529386 25:26982725 <i>LOC100847190 – ZNF688</i>	F, ACTAAAGATCCCACGTGCTA	500	This study
	R, GTCTTACTACTGTCCCCACA		
	P1, [R6G]AAGGTGCCACAGCCAGA[BHQ1]		
	P2, [ROX]AGGTGCCGCAGCCA[BHQ2]		
rs43038601 18:57032285 <i>TRNAG-UCC – TRNAG-UCC</i>	F, TGATAACACGTACA+GAGT	180	This study
	R, CAATAAGGCGATTTCGTGG		
	P1, [R6G]TGGTGTCTCGGTTGC[BHQ1]		
	P2, [ROX]TGTCTAGGTTGCTTTACTG[BHQ2]		
rs41255693 26:21272422 <i>SCD1</i>	F, CCCTTATGACAAGACCATCAACC	90	[18]
	R, GACGTGGTCTTGCTGTGGACT		
	P1, [FAM]CTTACCCACAGCTCCCA[BHQ1]		
	P2, [HEX]TACCCGCAGCTCCC[BHQ1]		

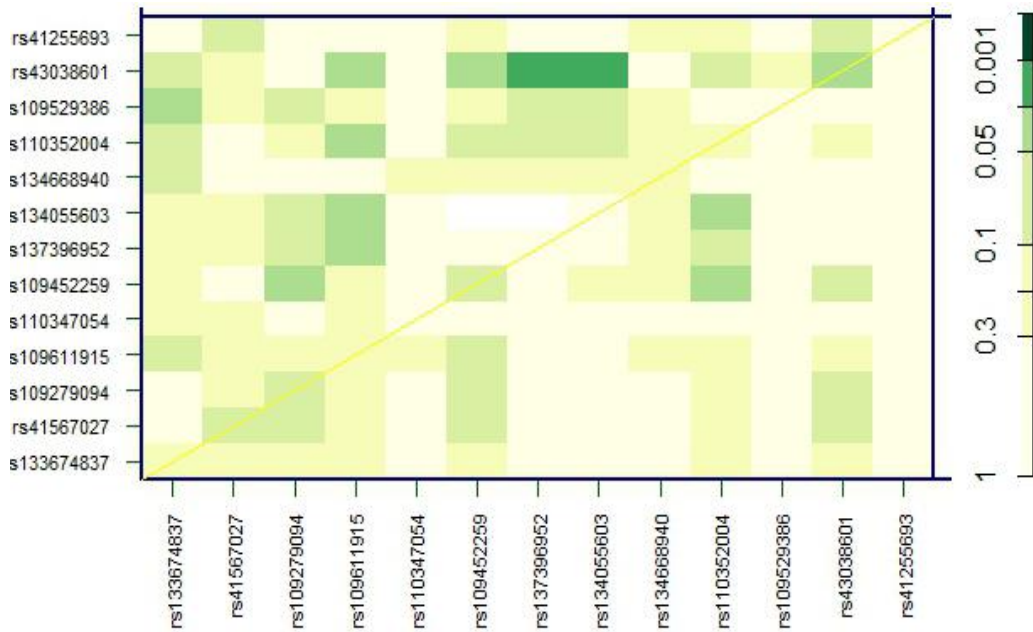


Fig. 1. Screening for the presence of epistatic and additive interactions among polymorphisms in relation to sacrum height parameter inheritance in Holstein cows

A statistically significant epistatic interaction was found for rs134055603 polymorphism. This SNP has a significantly positive contribution to sacrum height only in the presence of A* allele in a genotype identified by rs43038601 polymorphism, while its absence (the combination of G/G and C/C genotypes identified by rs134055603 and rs43038601, respectively) has a negative effect on the phenotype (Fig. 2). It is worth noting that the number of specimens of A/A genotype identified by rs43038601 polymorphism for each of the three combinations was ≤ 3 . Therefore, interpretation of these genotype

combinations is impossible. For each of the other six combinations, the sample size was ≥ 10 .

Epistatic interactions of opposite nature for 134055603 with rs137396952 polymorphism were found. The combination of A* allele in genotype of rs134055603 and the C* allele in genotype of rs137396952 had a positive effect on sacrum height (Fig. 3). It is worth emphasising that the number of specimens with A/A genotype identified by rs43038601 polymorphism for each of the three combinations was ≤ 3 .

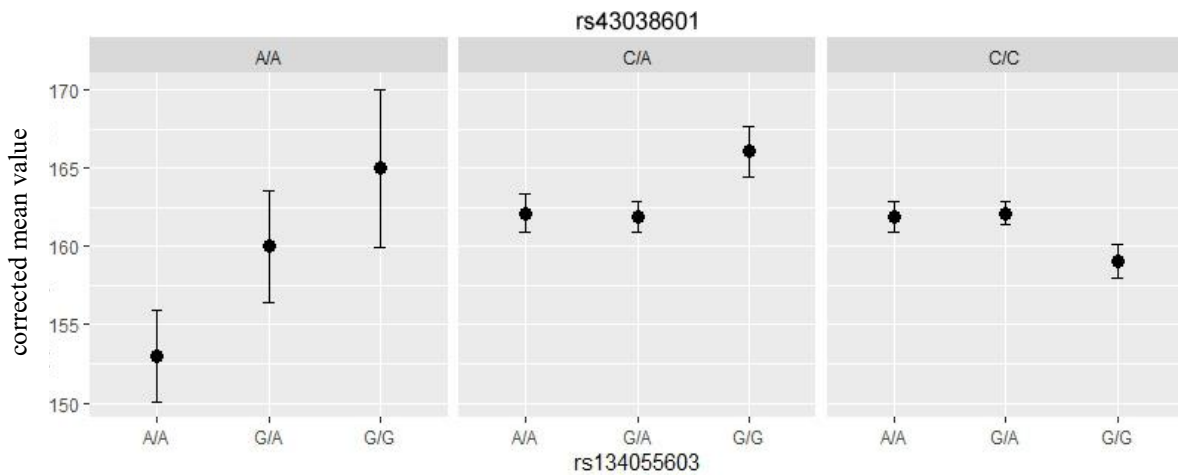


Fig. 2. Facet matrix for the pooled samples of Holstein cows in relation to sacrum height for rs134055603 and rs43038601 epistatic interaction. Corrected $R^2 = 0.0923$. F-test = 2.957. p-value < 0.0043

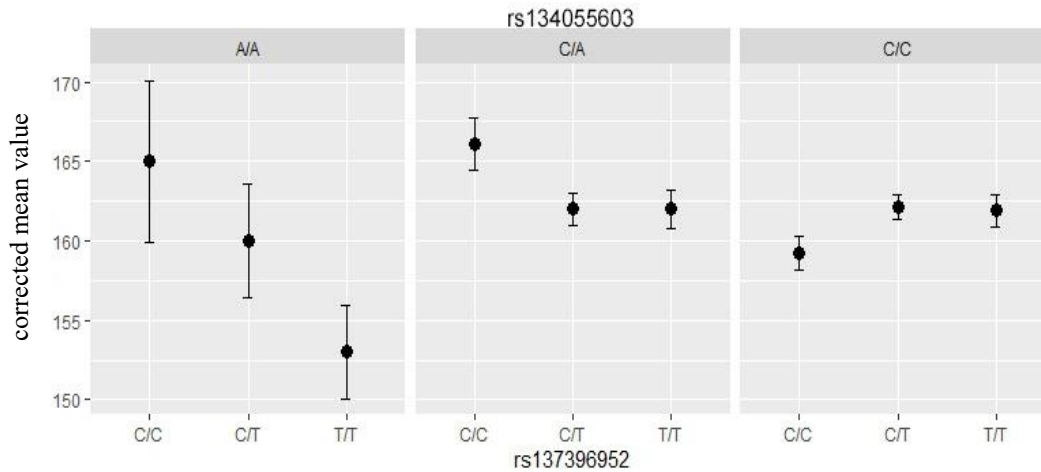


Fig. 3. Facet matrix for the pooled samples of Holstein cows in relation to sacrum height for rs134055603 and rs137396952 epistatic interaction. Adjusted $R^2 = 0.0902$. F-test = 2.909. p-value < 0.0049

Discussion and Conclusion. During the study conducted in 155 Holstein cows, the mean values and standard deviations for sacrum height (161.7 ± 5.3 cm), hip width (41.1 ± 3.9 cm), and ischial tuberosity width (22.4 ± 2.1 cm) were determined. Genotyping of 13 polymorphisms was conducted for each specimen. It was revealed that in Holstein cows, genotyping of rs109452259 requires negative selection by C* allele to increase the hip width. Epistatic interaction of rs134055603 and rs43038601, probably of synergistic nature for G/G and C/A genotypes respectively, was identified. Epistatic interaction of rs134055603 and rs137396952, probably of synergistic nature for the C/A

and C/C genotypes respectively, was identified. The data obtained during the study are significant for the observed-level statistical interaction; for confirmation of the biological mechanisms, further functional studies are required.

The potential limitations of the study include possible influence of the unaccounted confounding factors, such as, variability of the studied groups of animals by age, live body weight, lactation number, and origin (of an agricultural enterprise). The above mentioned limitations could have influenced the results obtained and their subsequent interpretation.

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About the Authors:

Maksim V. Bytov, Postgraduate Student, Junior Research Associate of the Genomic Research and Animal Breeding Department, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str, P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), bytovmaks@mail.ru

Vladlena D. Zubareva, Junior Research Associate of the Genomic Research and Animal Breeding Department, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str., P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), zzub97@mail.ru

Alexander S. Krasnoperov, Cand.Sci. (Veterinary), Head of the Laboratory of Immunology and Pathobiochemistry, Ural Research Veterinary Institute, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str., P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), marafon.86@list.ru

Natalia A. Bezborodova, Cand.Sci. (Veterinary), Head of the Genomic Research and Animal Breeding Department, Ural Research Veterinary Institute, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str., P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [ORCID](#), [Scopus ID](#), n-bezborodova@mail.ru

Marina A. Pechenkina, Laboratory Assistant of the Genomic Research and Animal Breeding Department, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str., P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), pn190503@mail.ru

Olga V. Sokolova, Dr.Sci. (Veterinary), Director of the Ural Research Veterinary Institute, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences (112a, Belinsky Str., P.O. Box 269, Ekaterinburg, 620142, Russian Federation), [SPIN-код](#), [Researcher ID](#), [Scopus ID](#), nauka_sokolova@mail.ru

Claimed Contributorship:

MV Bytov: development of methodology, formal analysis, preparing the draft of the manuscript.

VD Zubareva: conducting experiments and data collection, preparing the draft of the manuscript.

AS Krasnoperov: conducting experiments and data collection.

NA Bezborodova: conducting experiments and data collection.

MA Pechenkina: manuscript editing and refining.

Sokolova OV: manuscript editing and refining.

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All authors have read and approved the final manuscript.

Об авторах:

Максим Владимирович Бытов, аспирант, младший научный сотрудник отдела геномных исследований и селекции животных Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), bytovmaks@mail.ru

Владлена Дмитриевна Зубарева, младший научный сотрудник отдела геномных исследований и селекции животных Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), zzub97@mail.ru

Александр Сергеевич Красноперов, кандидат ветеринарных наук, заведующий лабораторией иммунологии и патобиохимии Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а), [SPIN-код](#), [ORCID](#), [Researcher ID](#), [Scopus ID](#), marafon.86@list.ru

Наталья Александровна Безбородова, кандидат ветеринарных наук, заведующий отделом геномных исследований и селекции животных Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а), [SPIN-код](#), [ORCID](#), [Scopus ID](#), n-bezborodova@mail.ru

Марина Андреевна Печёнкина, лаборант отдела геномных исследований и селекции животных Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а),

Ольга Васильевна Соколова, доктор ветеринарных наук, руководитель Уральского научно-исследовательского ветеринарного института, Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук (620142, Российская Федерация, г. Екатеринбург, а/я 269, ул. Белинского, 112а), nauka_sokolova@mail.ru

Заявленный вклад авторов:

М.В. Бытов: разработка методологии, формальный анализ; подготовка первоначального варианта рукописи.

В.Д. Зубарева: проведение экспериментов и сбор данных; подготовка первоначального варианта рукописи.

А.С. Красноперов: проведение экспериментов и сбор данных.

Н.А. Безбородова: проведение экспериментов и сбор данных.

М.А. Печёнкина: редактирование и доработка рукописи.

О.В. Соколова: редактирование и доработка рукописи.

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