ANNOTATION

dissertation work by Shormanova Marzhan Muratovna on the topic «Identification of genetic variants of TNP in breeding bulls and assessment of sperm fertility», submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D120100 – «Veterinary Medicine»

Relevance of the research topic. An accessible method for increasing the genetic potential of the population, increasing milk and meat productivity of cattle is the use of breeding highly productive bulls for herd reproduction. Therefore, at present, much attention is paid to the issues of selection, selection of breeding bulls with high parameters of reproductive function, which are not carriers of harmful latent mutations. In the dissertation work, SNP polymorphisms (g.269 G → A, g.1536 C → T SNP) in the coding part of the TNP2 gene, polymorphism in the exonic part of the SPEF2 gene, which are associated with the indicators of the reproductive function of bulls, the expression of these genes control the processes of spermatogenesis, were studied as DNA markers of reproductive function. Analysis of world literature shows that the use of SNP polymorphisms allows identifying individuals with the desired genotype, which have a higher reproductive capacity.

Recently, there has been a tendency in the world to reduce the reproductive function of high-yielding cows for two reasons: increased milk productivity, high metabolic rate, which is accompanied by decreased fertility in cows. The second very important reason is an increase in heterozygous carriers of hidden harmful mutations that arose as a result of point mutation, deletion or insertion; in cows, such anomalies are accompanied by impaired embryonic development, hidden abortion often occurs, causing great economic damage to dairy cattle breeding.

Currently, the development of molecular genetic methods for diagnosing carriers of harmful mutations that are accompanied by embryonic mortality in cows and reduce the yield of young animals is especially relevant. In this work, methods for diagnosing such hereditary anomalies in the Holstein breed as fertility haplotypes HH4, HH5, HCD and subfertility syndrome in bulls were optimized and developed. For the first time, the method of ultrasound scanning of the reproductive organs using an endorectal sensor was used to determine the morphological state of the accessory sex glands in bulls.

Gene loci are known, the alleles of which have a reliable effect on the quality of sperm, on fertilization. In veterinary andrology, it is important to use hormonal drugs to stimulate sperm productivity of breeding bulls in large breeding centers. To improve reproductive function, drugs that have a stimulating effect on the process of spermatogenesis are successfully used.

The aim of the dissertation Study of the prevalence of genetic variants in the loci of the TNP2, SPEF2 genes, study of the influence of alleles of these genes on the reproductive function of bulls of producers belonging to the breeding center of «Asyl Tulik», «Taurus», «Asyl» in Taldykorgan, Zhetysu region, «Sarkand-Agro» in Yenbekshikazakh district, Almaty region, «Bayserke-Agro» in Talgar district, monitoring of genetic defects, identification of heterozygous carrier bulls.

Research objectives:

1. Conducting genotyping of stud bulls belonging to «Asyl», private livestock farm «Sarkand-Agro», multidisciplinary farm «Baiserke-Agro» using the PCR-RFLP method at the TNP2 gene locus, studying the genetic structure of stud bulls of the studied population, studying the frequency of gene alleles, gene balance;

2. Determination of the genotype of breeding bulls belonging to the breeding centers and farms "Asyl Tulik", "Asyl", "Sarkand-Agro", "Baiserke-Agro" by the locus of the SPEF2 gene, study of the genetic structure of bulls of the studied population, study of the frequency of gene alleles, gene balance;

3. Study of the relationship between the indicators of sexual activity of bulls, libido, sexual activity of bulls during one season and genetic characteristics of the TNP2 gene locus in production conditions on the farms "Sarkand-Agro" and "Baiserke-Agro";

4. Determination of the influence of the SPEF2 gene alleles on the sperm productivity indicators of bulls produced by «Asyl Tulik», ejaculate volume, sperm concentration, and the number of active sperm;

5. Conducting genetic monitoring for the carriage of heterozygous carriers of the fertility haplotypes HH4, HH5, HCD and subfertility syndrome in bulls of «Asyl Tulik», limited liability partnership «Taurus», identifying heterozygous carriers;

6. Studying the influence of negative factors on the reproductive function of bulls of the breeding farms «Sarkand-Agro», «Bayserke-Agro» and introducing optimal schemes for stimulating the reproductive function of bulls, studying their effectiveness.

Materials and methods of research. Frozen blood samples with anticoagulant and cryopreserved samples of semen of bulls were used as the material for the study. DNA was isolated from biological samples using commercial kits and the classical phenol method. The quality of the isolated DNA was assessed by horizontal electrophoresis and by measuring the DNA concentration. Modeling of molecular genetic research methods: collection of biological samples, DNA extraction, primer design, determination of amplification conditions, visualization of PCR results by horizontal electrophoresis, determination of the genotype of samples using a gel documentation system.

The work was carried out using modern molecular genetic research methods. To assess the quality of the obtained DNA, the method of horizontal electrophoresis in 0.8% agarose gel was used, this method allows to indirectly determine the concentration of DNA and the degree of DNA fragmentation, which is also an important criterion for assessing the quality of DNA. The concentration of DNA was measured using the nanodrop 2000 device, the concentration of DNA and the degree of DNA purification were determined, which was above 0.75.

Computer programs Primer 3, Primer 1, a calculator for determining the $\chi 2$ value, and information from the NCBI website were also used. In the dissertation work, the sequences of primers described in the literature and our own primers were used for genotyping of DNA samples. For genotyping of breeding bulls for carriage of subfertility syndrome in bulls, the Tetra-Primer ARMS-PCR reaction method was

used, which has an advantage over the classical PCR-RFLP analysis, excluding the use of restriction enzyme.

The experimental part of the work was carried out on bulls-producers of the breeding center of JSC "Asyl Tulik", gnotyping of DNA samples was carried out on a large sample. In order to study the influence of alleles of the studied genes on sperm productivity, the following parameters of the reproductive function of bulls were determined, whose genotypes were known for the locus of the TNP2 and SPEF2 genes. Based on the analysis of the results of obtaining sperm, cryopreservation of sperm, sale of frozen sperm of 25 breeding bulls of the breeding center of JSC "Asyl Tulik", 5 bulls with high reproductive function were identified. The largest volume of sales of sperm doses of the specified bulls, the number of farms that used the sperm of the top bulls for reproduction. It should be noted that the bulls with high sales volumes and the number of farms were bulls with a heterozygous TG genotype for the SPEF2 gene locus. Based on the results of the analysis of the reproductive function of bulls for the period from 2016 to 2021, the best bulls were identified, the bull "Sokol" of the Simmental breed, the bull "Champion" of the Kazakh white-headed breed. The results obtained suggest that DNA markers can be used to predict the sperm productivity of bulls.

To assess the morphological state of the accessory sex glands in bulls, the prostate and vesicular glands, an ultrasound scanning method was used using PU2200 Vet and Mindray Z5 Vet ultrasound devices.

It should be noted that ultrasound examination in veterinary practice is mainly used to determine pathological changes in parenchymatous organs, in the reproductive organs of female farm and domestic animals. Analysis of the use of ultrasound scanning shows that this method is not used in practice in veterinary andrology. In the dissertation work, the ultrasound scanning method of the accessory sex glands in bulls was used for the first time to determine morphological changes. The most accessible for ultrasound examination are the accessory sex glands: the prostate and vesicular glands. Ultrasound examination allows you to determine the morphological state of the accessory sex glands, the presence of pathological changes, especially adhesions. Sufficiently good quality sonograms were obtained, where the structure of the prostate and vesicular glands is visible.

Determination of sexual activity of bulls, sexuality, libido level was carried out by observation method, microscopic indices of ejaculate quality were determined using microscope and AFS 500 device. Testing of efficiency of methods of stimulation of reproductive function of bulls was carried out in conditions of breeding farms, efficiency of used methods of stimulation was established.

In the dissertation work, the following preparations were used to stimulate the reproductive function: surfagon, tissue preparation ASD2. The schemes for stimulating the reproductive function of bulls were optimized taking into account the characteristics of the spermatogenesis process, which lasts 45-60 days. The spermatogenesis process is a complex biological process, and this process can be improved by hormonal effects on the body of bulls and also by increasing metabolic processes in the body as a whole. To identify point mutation in coding part of gene TMEM95 (bull subfertility syndrome) method of Tetra-Primer ARMS-PCR reaction

was used. Schemes for stimulating the reproductive function of breeding bulls have been developed, and the results of stimulating sperm productivity in breeding bulls have been analyzed. The doctoral student has publications on the use of the electroejaculation method for obtaining sperm from breeding bulls in farm conditions.

Scientific results, their validity and novelty. The scientific novelty of the dissertation is the determination of the level of genetic polymorphism at the loci of the TNP2 genes (SNP polymorphisms g.269 G→A SNP, g.1536 C→T) and SPEF2 in the studied bulls, the study of the influence of the alleles of these genes on the indices of sperm productivity. Genetic polymorphism was determined for all studied gene loci in the studied bulls. As a result of amplification of the corresponding gene fragments, amplification products were obtained; the sizes of the obtained PCR products are clearly distinguished on the electrophoregram. Restriction enzymes were used to identify the alleles of the TNP2 and SPEF2 genes. Currently, the only accessible and inexpensive method for identifying gene alleles is the PCR-RFLP analysis method. As an alternative method of identification in molecular genetics, the Real Time PCR method is used, which is a more expensive method for identifying gene alleles. For the first time, heterozygous carriers of the fertility haplotypes HH5, HCD and subfertility syndrome were identified in breeding bulls, the Tetra-Primer ARMS-PCR diagnostic method was developed. The proof of the scientific novelty of the dissertation is the obtained 4 patents for the invention of the Republic of Kazakhstan, 1 patent for a utility model, the publication of the research results in the highly rated journal Q2, percentile 71.

Compliance with the main directions of scientific development or state programs. The dissertation work was completed within the framework of the scientific project of the Ministry of Science and Higher Education of the Republic of Kazakhstan «Development of molecular genetic methods for detecting hidden mutations in cattle and managing the process of eliminating hereditary anomalies», IRN AP09057988, implementation period 2021-2023. The doctoral student prepared an application on the topic: “Study of the influence of gene alleles on sperm productivity and the study of sperm fertility of breeding bulls” AP25793505 for participation in the competition “Zhas Galym” and the decision of the NSC was approved.

Description of the doctoral student's contribution to the preparation of each publication.

Based on the results of scientific research, the doctoral student published 4 articles under the supervision of scientific consultants, including 3 articles in the journals of the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, 1 article in a journal included in the Scopus database. Article in the journal «Reproduction in Domestic Animals», article title «Development of alternative diagnosis of HH1, HH3, HH5 and HCD fertility haplotypes and subfertility syndrome in cattle», 2024, 59(1), e14533. In order to study the influence of alleles of the TNP2 and SPEF2 genes on sperm productivity and ejaculate parameters, a large amount of work was carried out to collect information for the period from 2016

to 2022, analyzing the records of the sperm collection log of breeding center No. 1, taking into account the following parameters: volume of ejaculate obtained, sperm motility after obtaining ejaculate and after sperm dilution before cryopreservation, the number of sperm doses obtained as a result of dilution.

Volume and structure of the dissertation. The dissertation is presented on 122 pages of computer text and consists of an introduction, literature review, research materials and methods, results of own research, discussion of research results, conclusion, proposal for production, list of used sources, appendices. The dissertation is illustrated with 21 tables, 20 figures. The list of references includes 180 sources.