ABSTRACT

dissertation work of Asset Abdymaratovich Turgumbekov on the topic «Increasing the reproductive function of cows based on a study of the dynamics of growth of the dominant follicle and genotyping at the Erα gene locus», submitted for the degree of doctor of philosophy (PhD) in the specialty 6D120100 – "Veterinary medicine"

Relevance of the research topic. In the world over the past 15-20 years, there has been a trend towards a decrease in the reproductive function of cows due to an increase in milk productivity as a result of intensive breeding work to increase milk yield. It is known that the heritability coefficient of the phenotypic trait of the reproductive function of cows is low and amounts to only 0.02. Thus, the study of the characteristics of follicle growth, the study of the mechanism of ovulation, the development of optimal schemes for synchronizing the estrous cycle in dairy cows and their theoretical justification are of great practical importance. An analysis of domestic literature shows that Kazakhstani scientists have not conducted studies of the growth characteristics of dominant and subdominant follicles in cows, although foreign scientists have proven that during the estrous cycle in cows there are two or three waves of growth of dominant follicles. Modern diagnostic methods, such as ultrasound scanning of the ovaries and the use of Doppler ultrasound, make it possible to determine the dynamics of follicle growth, study the rate of follicle growth, determine the size of dominant and subdominant follicles, and determine the degree of vascularization of the corpus luteum. In developed countries, genomic selection is used to predict reproductive function, which makes it possible to identify animals with the desired genotype and increases the yield of calves per 100 cows. However, in our country, the implementation of genomic selection technology is currently limited due to the complexity of collecting phenotypic data, weak infrastructure, and lack of a reference population. Therefore, the study of DNA markers of reproductive function in cattle is an urgent problem in veterinary science. There are multiple SNP polymorphisms, the alleles of which have an associative effect on the reproductive function of cows. Most foreign scientists use SNP polymorphisms at the loci of the following genes – estrogen receptor (ERα) and differentiation growth factor (GDF9) as DNA markers of reproductive function. Breeding animal genotyping technology allows the identification of individuals with a desired genotype that have an advantage over other genetic variants. It should be noted that often in practice, synchronization and stimulation of the estrous cycle in cows is carried out frontally, without taking into account the stage of the reproductive cycle, without taking into account the growth dynamics of dominant and subdominant follicles. The development of optimal schemes for synchronizing the estrous cycle in cows, taking into account the dynamics of the growth of dominant follicles, is of practical importance for dairy farms.

The purpose of the dissertation work is to study the dynamics of the growth of dominant and subdominant follicles in cows during the estrous cycle, determine the number of waves of follicle growth, study changes in the concentration of the

hormone estradiol and the associative effect of alleles of the *ERα,* GDF9 genes on the reproductive function of cows.

Research objectives:

- study of the dynamics of the growth of dominant and subdominant follicles in Holstein cows using ultrasound scanning of the ovaries with an interval of 48 hours in the conditions of the Baiserke-Agro LLP dairy farm during one estrous cycle;
- determination of the amount of the hormone estradiol in cows of the study group during the preovulation period and in randomly selected cows using ELISA analysis;
- DNA isolation from the studied group of cows, determination of the quality of isolated DNA by horizontal electrophoresis and by measuring DNA concentration using a nanodrop device;
- creation of an experimental group of animals in the amount of 120 heads of the breeding farm of Baiserke-Agro LLP and collection of biological material, DNA isolation, genotyping of cows at the *Erα* gene locus using PCR-RFLP analysis;
- obtaining the results of genotyping cows at the estrogen receptor gene locus Erα, identifying genetic variants and studying the effect of alleles of this gene on reproductive function;
- taking blood samples from experimental groups of the Baiserke-Agro LLP dairy farm, extracting DNA from blood samples, genotyping cows at the GDF9 gene locus SNP A625T/DRAI polymorphism using PCR-RFLP analysis;
- obtaining the results of genotyping cows at the GDF9 gene locus, identifying genetic variants and studying the influence of alleles of this gene on the reproductive function of cows;
- study of the effectiveness of various schemes for synchronizing the estrous cycle in cows of the dairy farm of Beiserke-Agro LLP.

Materials and research methods.

Experimental work to study the dynamics of growth of dominant and subdominant follicles was carried out on Holstein cows of foreign selection with a milk productivity of 8500-9000 kg per lactation in the conditions of the breeding farm of Bayserke-Agro LLP in the Talgar district of the Almaty region using ultrasound scanning of the ovaries with an interval of 48 hours using devices PU2200 Vet and Mindray Z5 Vet. Determination of the content of the hormone estradiol in blood serum samples from cows during the preovulatory period was carried out in the laboratory of the Department of Obstetrics, Surgery and Biotechnology of Reproduction of KazNAIU using an ELX808 ELISA analyzer (microplate reader) using a commercial kit from the Russian company ImmunoFA Estradiol. Genotyping of DNA samples for the Erα and GDF9 gene loci was carried out in the laboratory of «Green Biotechnology and Cell Engineering» of the Kazakh-Japanese Innovation Center of KazNAIU.

To genotype cows for the estrogen receptor gene loci Erα, GDF9 SNP A625T/DRAI, frozen animal blood was used as material. Blood was taken from the jugular vein, sometimes from the tail vein, into 2.0 ml EDTA vacuum tubes.

Blood samples were stored in a freezer, DNA extraction from blood samples was carried out in the laboratory of the Department of Obstetrics, Surgery and Biotechnology of Reproduction using the phenol method, also using a commercial DNA sorb-B kit, produced in Russia. The quality of isolated DNA was determined by horizontal electrophoresis, the concentration of DNA samples was measured using a NanoDrop™ 2000 microspectrophotometric analyzer, the amount of nucleic acids in the samples and the degree of DNA purification were determined. For genotyping DNA samples of 120 Holstein cows at the Erα and GDF9 gene loci, forward and reverse primers were used, the sequences of which were taken from literary sources. However, to eliminate errors, the Erα and GDF9 gene sequences were analyzed and the optimal primer annealing temperatures were determined. Amplification of the desired fragment of the Erα and GDF9 genes was carried out using amplifiers produced by the German company Eppendorf and SimpliAmp. Horizontal electrophoresis and a gel documentation system were used to visualize the results of the polymerase chain reaction and to identify genetic variants of DNA samples. Detection of genetic variants at the studied gene loci was carried out using the restriction method of PCR product. The following reagents were used: 10x Taq Buffer KCL, dNTP mixture (25 mM), Taq DNA Polymerase (recombinant) 5U/μl, 25 mM MgCl2, for horizontal electrophoresis we used 1XTAE buffer, agarose, ethidium bromide, gel dye, DNA markers. An analysis of the reproductive function of cows with different parameters of the reproductive function depending on the genotype of the animals was carried out, and the associative effects of alleles of the Erα, GDF9 genes on the reproductive ability of cows were established. Optimal schemes for synchronizing the estrous cycle in cows have been developed depending on the diameter of the dominant follicles; the highest results of artificial insemination were in cows in which dominant follicles with a diameter of 5-8 mm were detected on the day of synchronization. To synchronize the estrous cycle in the postpartum period, the OvSynch synchronization scheme was used, and for synchronization in cows with a long period of anestrus, another synchronization scheme was used - PreSynch-OvSynch. To assess the impact of genetic variants at the Erα, GDF9 gene loci on reproductive function, a criterion was used - the index of non-return of cows after artificial insemination on the 58th day.

Main provisions submitted for defense:

- results of ultrasound scanning of the ovaries of Holstein cows in a dairy farm with an interval of 48 hours, about the dynamics of growth of dominant and subdominant follicles, the growth rate of dominant follicles, the number of follicle growth waves, the duration of the growth phase of dominant follicles, the diameter and volume of dominant follicles;

- about the results of genotyping cows at the estrogen receptor gene loci *Erα,* GDF9 using molecular genetic methods of PCR, PCR-RFLP analysis, the quality of isolated DNA, primer design, restriction conditions;

- the prevalence of genetic variants at the estrogen receptor gene locus Erα in cows of the dairy farm of Baiserke Agro LLP, a violation of gene balance, the influence of alleles of the gene under study on the reproductive function of cows;

- the prevalence of genetic variants at the GDF9 gene locus in cows of the dairy farm of Baiserke Agro LLP, a violation of gene balance, the influence of alleles of the gene under study on the reproductive function of cows;

- on the results of the analysis of the influence of various genetic variants at the *Erα,* GDF9 gene loci on the reproductive function of cows, the insemination index of cows, the index of non-return of cows after artificial insemination on the 58th day;

- on the effectiveness of synchronization of the estrous cycle in cows, taking into account the dynamics of the growth of dominant and subdominant follicles in cows.

Scientific results, their validity and novelty.

The scientific novelty of the dissertation work is the study of the dynamics of growth of dominant and subdominant follicles during the estrous cycle in cows; for the first time in Holstein cows, the number of waves of follicle growth was determined (two and three waves of follicle growth), the duration of the growth wave of dominant follicles was studied, the role of hormone concentration was determined estradiol in the process of folliculogenesis, the hypothesis of ensuring the growth of one dominant follicle by a population of subdominant follicles has been proven, the Doppler ultrasound scanning method has been successfully used to determine the degree of vascularization of the corpus luteum of the reproductive cycle, the synchronization scheme of the estrous cycle in cows has been optimized, taking into account the growth of dominant follicles, the associative influence of *Erα* gene alleles has been studied, GDF9 on the reproductive function of cows.

The results of the study of the dynamics of growth of dominant and subdominant follicles make it possible to develop scientifically based schemes for synchronizing the estrous cycle in cows. Determining the degree of vascularization of the corpus luteum is of practical importance; it allows one to predict the development of pregnancy, since well-defined vascularization of the corpus luteum indirectly indicates high secretion of the hormone progesterone, which supports pregnancy in cows. The dissertation work determined the influence of alleles of the Erα, GDF9 genes on the reproductive function of cows. Thus, at the estrogen receptor gene locus, cows with the homozygous GG genotype had higher reproductive function parameters. During the dissertation work, such modern research methods were used as ultrasound scanning of the ovaries, DNA certification of cows, PCR methods, PCR-RFLP analysis, processing of the results obtained, determination of the $\chi 2$ value using the Hardy-Weiberg formula, study of gene balance, actual and theoretical distribution genetic variants at the *Erα*, GDF9 gene loci in the studied animal population.

Based on the results of ultrasound scanning, good quality sonograms were obtained, a clear record of the results of the ultrasound examination was carried out, the diameters, location of dominant and subdominant follicles, and the number of waves of follicular growth were determined, which indicates the reliability of the results obtained. Thermo Fisher Scientific reagents were used to genotype DNA samples at two gene loci.

Compliance with the main directions of scientific development or government programs.

The dissertation work was carried out within the framework of the scientific projects of the Ministry of Education and Science of the Republic of Kazakhstan «Intensification of the selection process in livestock breeding based on the use of cellular reproductive technologies», registration number No. 0115RK00728, implementation period 2015-2017, the scientific project of the Ministry of Education and Science of the Republic of Kazakhstan «Monitoring of breeding animals of meat production for the carriage of hidden genetic anomalies», IRN AP15473095, implementation period 2022-2024.

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

Based on the results of scientific research, the doctoral student prepared and published 4 articles under the guidance of scientific consultants, including 3 articles in journals of the Committee for Quality Assurance in Science and Higher Education, 1 article in a journal included in the Scopus database.

1 article in the journal «International Journal of Veterinary Science», article title «Results of Ultrasound Studies of the Growth Dynamics of Dominant, Subdominant Follicles and Determination of Estradiol Concentration in the Preovulatory Period in Cows», ISSN 2304-3075, 2305-4360, 2023, Vol.12, No. 5, 36 pp. 680-689, Scopus

3 articles in publications recommended by the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan:

in the Journal – «3i: intellect, idea, innovation - intelligence, idea, innovation» – Kostanay University named after Akhmet Baitursynov, 2023. – No. 2. - P. 147-154.

in the Journal «Gylym zhane Bilim», West Kazakhstan Agro-Technical University named after Zhangir Khan, 2023. - No. 2-2 (71). – pp. 121-129.

in the Journal «Gylym zhane Bilim», West Kazakhstan Agro-Technical University named after Zhangir Khan, 1-bolim. 2023. – No. 4-1 (73). - pp. 182-193.

Scope and structure of the dissertation. The dissertation is presented on 125 pages of computer text and consists of an introduction, literature review, materials and research methods, results of own research, discussion of research results, conclusion, proposal for production, list of sources used, applications. The dissertation is illustrated with 23 tables and 26 figures. The list of references includes 203 sources.