

ABSTRACT

the dissertation work of Sarybaev Yktyiar Ualibekovich on the topic «Genoty of cows by LHCGR, FSHR loci and study of the level of ovulation depending on the expression of the genes under study» submitted for the degree of Doctor of Philosophy (PhD) speciality 6D120100 - Veterinary medicine

Relevance of the research topic. One of the main causes of infertility of cows in our country are pathologies found in the ovaries. Among breeding cows, ovarian pathologies account for the majority of gynecological diseases.

Many scientists have provided data and scientifically discussed the process of ovulation in the ovaries of breeding cows based on ultrasound examinations and analyses of FSH, LH, and estrogenic hormones in the blood. As a result, the importance of the hormones FSH and LH in the formation of the corpus luteum and the dynamics of follicle growth has been studied. This research allows for the timely detection of pathological changes in the reproductive system, thereby minimizing economic losses. Repeated examination of the pathological changes that have formed is not only a waste of time but also creates conditions for the exacerbation of the disease.

However, the modern research we are conducting will undoubtedly contribute to the reproduction of pedigree and highly productive, disease-resistant animal breeds in our country.

Today, in our country, due to the growth of breeding stock, livestock specialists are faced with the task of giving importance to the quality of the young stock they receive, rather than its quantity. In this context, various pathologies found in the genitals of the breeding stock on many farms hinder the stable productivity of cows

As a result, a number of livestock farms in the republic do not produce 20-30% of the estimated livestock. This situation is more common in dairy farms, as technological processes for the reproduction of products are disrupted, as well as the breeding stock remains untimely and has not been inseminated for a long time.

Based on data from the scientific literature, domestic and foreign scientists have presented the results of studies of the hormones FSH and LH, which have a direct effect on the course of ovulation in the ovum under normal conditions.

It should be noted that the passage of the ovulation process under normal conditions still requires special observations and research. In the maternal organ, the ovulation process is influenced by other factors besides those mentioned above.

Cows with normal reproductive function that are not bred after regular fertilization attempts at least three times lead to significant economic losses on dairy farms.

The purpose of the dissertation research. The main purpose of the scientific research is the genotyping of Holstein cows according to the loci LHCGR and FSHR, and determining the level of ovulation in the research group.

This includes increasing their reproductive function and the level of fertilization and calving.

Research objectives:

1. To sample blood and isolate DNA from cows in the research group, investigating the concentration and quality of DNA samples using a NanoDrop 2000.

2. To examine DNA samples from 155 cows in the research group at Bayskerke-Agro LLP using PCR for the LHCGR and FSHR loci.

3. To study the relationship between genetic variants at these loci and reproductive function after obtaining the genotyping results for the LHCGR and FSHR loci of cows in the research group.

4. To study changes in the ovaries of Holstein cows during the sexual cycle in the research group under the conditions of the dairy farm "Baiserke-Agro" using ultrasound.

5. To study the concentration of FSH and LH hormones in the blood serum of cows in the study group using the ELISA method during ovulation and at different stages of the reproductive cycle.

Materials and methods

155 heads of Holstein dairy cows were selected as subjects for the dissertation research. Specialists and veterinarians were assigned the responsibility of collecting blood samples and conducting further analysis. The research team also included laboratory analysts and other necessary specialists. All members of the research group were briefed on the procedures, protocols, and safety measures for working with animals.

Research protocols have been developed, and blood samples of 155 heads of Holstein dairy cows have been obtained. The research work on DNA extraction from blood was carried out by the phenol-chloroform method in the Laboratory of Molecular Cytogenetics of the All-Russian Scientific Research Institute of Genetics and Breeding of Farm Animals in St. Petersburg. Verification and assessment of DNA quality and concentration were carried out in the laboratory department "Green Biotechnology and Engineering of the Young" of the Kazakh-Japanese Innovation Center at the Kazakh National Agrarian Research University.

The safety and proper transportation of the obtained samples to the laboratory was ensured. In addition, studies of the female genital glands (transrectal, ultrasound) of cows in this farm, Holstein frieze breeding cows of 3-5 years of age weighing 350-500 kg, and dairy direction were additionally conducted.

According to several domestic scientists, ovarian diseases are considered the primary among the sexual pathologies affecting breeding cows.

Among the listed pathologies, disorders of the functional function of the ovaries are most common, namely: hypofunction (42.3%), persistent corpus luteum (34.6%), and ovarian vesicle (23.1%).

As we know, the FSHR and LHCGR genes play a crucial role in the release of gonadotropins, significantly impacting the reproductive function of breeding stock. Therefore, it is imperative to select cows for genotyping at the FSHR and

LHCGR loci using ultrasound examination of the livestock.

Previously, we relied on traditional methods to identify gynecological pathologies within our herd. However, these methods are time-consuming and do not always yield accurate results regarding gestation rates. In contrast, the modern method employed in our research is ultrasound.

For the study of cows, along with special methods, a PU-2200v ultrasound machine made in the USA was used. 155 heads of animals were subjected to ultrasound examination of 30 heads per day with a preliminary schedule.

On the first day, we separated the animals under study from the herd and moved them into a shed with specialized individual insulation. We prepared an ultrasound device and connected it to a power source. The monitor was then activated according to the program to assess the condition of the ovaries and uterus comprehensively.

Our research was conducted on three separate occasions: firstly, in the second half of October involving 50 cows; secondly, in the latter half of November with another 50 cows; and finally, in the first half of December, with a total of 55 cows. Ovarian pathologies stand out as a significant cause of infertility in cattle. During these critical periods, the reproductive health of the herd can be compromised, leading to potential delays in fertilization during the postpartum phase due to disruptions in genital gland processes and impaired egg maturation.

Diseases affecting the genital glands in cows often result in disturbances within the hormonal and endocrine systems. Clinically, these disturbances manifest as changes in the stages of the sexual cycle, impacting the overall reproductive health and performance of the animals.

Therefore, it is now understood that the use of transrectal and ultrasound methods for studying the female genital glands is crucial. These methods aid in selecting highly productive animals, identifying various pathological symptoms in the reproductive system of the herd, and implementing necessary therapeutic measures.

Furthermore, genotyping at the LHCGR and FSHR loci was conducted at the laboratory of green biotechnology and cell engineering at the "Kazakh-Japanese Innovation Center".

The scientific literature shows the importance of the LHCGR and FSHR genes in regulating the reproductive function of Holstein cows. In highly productive holstein cows of the SNP (Single nucleotide polymorphism) breed in the promoter and exon parts of the LHCGR and FSHR genes, polymorphisms have been reported to be associated with the onset of the first ovulation in the postpartum period.

Key propositions (validated scientific hypotheses and additional conclusions that contribute to the advancement of knowledge)

According to our study, animals with the CC genotype (promoter part) of the LHCGR, FSHR and (exon part) of the TT genotype had lower reproductive rates compared to other genotypic animals, in cows with such a genetic indicator, embryonic mortality often decreased in the first half of childbirth. The results of our study, which we received as immune infertility, are also presented in the

scientific literature (Voronina E. S., Petrov A.M., Serykh M. M. et al., 2002; Petrov A.M., 2009), confirms the data.

Description of the main results of the research

According to our study, a value was assigned to the fertility index of cows of the GG genotype of 36 heads, and the farm was asked to increase the number of cattle in this genotype.

The novelty of research work lies in several key aspects:

1. Molecular genetic study of the reproductive system of highly productive (meat, dairy) farm animals based on comprehensive analysis of scientific literature.
2. Fundamental investigation into the mechanisms of physiological and pathological processes occurring in the ovaries.
3. Study of gonadotropic and gonadal hormones that directly impact the productivity and reproductive system activity of animals.
4. Establishment of the ratio of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their functional activity in ovaries under physiological conditions.
5. Conducting genotyping of pedigree Holstein cows at FSHR and LHCGR loci using polymerase chain reaction from isolated DNA. We identified the prevalence of these loci in our genetic research group and discovered the associative effects of alleles at these gene loci on reproductive function.
6. Examination of LH and FSH receptor indicators of gonadotropins, which play a direct role in fertility during the sexual cycle. We also revealed the relationship between the expression of these studied genes and the level of ovulation.

This research contributes to a deeper understanding of genetic factors influencing reproductive function in farm animals, potentially leading to improved breeding strategies and enhanced productivity outcomes. The theoretical and practical importance of research work.

Significance of the research work

1. Enhanced Understanding of Gonadotropin Hormone Receptors: Through our research, we have deepened our knowledge of the parameters associated with gonadotropin hormone receptors LH and FSH. This includes insights into how these receptors influence the level of ovulation, linked to the expression of specific genes studied.
2. Integration into Scientific and Pedagogical Processes: The data and findings from our dissertation have been integrated into scientific and educational contexts, contributing to the training of veterinary specialists. Our research has enriched disciplines such as obstetrics, surgery, biotechnology of cultivation, and molecular genetic methods of gestation in veterinary medicine.
3. Practical Implications for Breeding and Reproductive Management: The results of our study, particularly concerning indicators of fertilization and calving in cows with the GG genotype, hold practical significance. These findings can inform and guide other researchers and practitioners in optimizing breeding strategies and reproductive management practices.

Suggested topics for research:

- Isolation, regulation, and distribution of gonadotropic hormones in cows.
- Characteristics and functions of LH and FSH gonadotropin receptors.
- Role and impact of LHCGR and FSHR genes on gonadotropic hormone regulation.
- Genotyping of cows using LHCGR and FSHR loci and the influence of gene alleles on reproductive function.
- Expression patterns of LHCGR and FSHR genes and their physiological roles during parturition.
- Physiological activities of hormones FSH and LH during pregnancy.;
- Articles on research work

Publications of the research work

6 papers have been published on the dissertation, including 2 articles are included in the Scopus database, the Open Veterinary Journal published the publication "Genotyping of cows by LHCGR, FSHR loci, and determination of the level of ovulation depending on the expression of the studied genes" ISSN: 2226-4485. 2023. Vol. 13(3): 352-357.(52 percentile) and on the topic "Spectrophotometric method for determining the quantity and quality of DNA in animal breeding" in the Homepage magazine: <https://sciencehorizon.com.ua> Scientific Horizons, (2024). Vol 27 (2), (28 percentiles). 31-42. UDC 577:636. DOI: 10.48077/scihor2.2024.31.

– 4 articles from the list of the Committee for control in the field of education and science of the Ministry of Education and Science of the Republic of Kazakhstan in republican scientific journals and collections of materials of the international scientific and practical conference. (2019-2020).

The volume and structure of the dissertation. The dissertation spans 108 pages of computer text, encompassing quantitative references, definitions, designations, and abbreviations. Its structure includes an introduction, review of scientific literature, independent research (the main focus), analysis of research results, conclusion, practical recommendations, list of references, and appendices. The dissertation incorporates 11 tables and 13 figures, and draws upon 170 printed publications.