

ANNOTATION
of the dissertation work of Junussova Raikhan Zheksenbaevna entitled
«Development methods for diagnosing genetic defects associated with
embryonic lethality and preventing gynecological pathologies in cows»,
submitted for the degree of Doctor of Philosophy (PhD) under the educational
program 8D09101—«Veterinary Medicine»

Relevance of the study topic. Identifying genetic alterations that lead to reduced reproductive capacity in farm animals, studying the molecular genetic mechanisms responsible for cattle fertility, and determining the causes associated with embryonic loss form the basis for the effective development of livestock farming. In this regard, whole-genome sequencing enables the identification of mutations that have a lethal effect on embryos, as well as genomic regions leading to economically significant problems, such as genetic abnormalities and developmental disorders. The results of fundamental research have shown that gamete maturation and embryonic development processes, whilst contributing to the formation of various organs and systems, also lead to the death of blastomeres and, consequently, to the formation of structural abnormalities and increased embryonic mortality.

There are many factors that reduce the reproductive capacity of cows, although the significance of each has varied over the years. Keeping cows on dairy farms is only viable if they consistently maintain high levels of productivity and growth. However, herd size affects their reproductive system and causes various functional changes, which manifest as hypofunction, delayed ovulation, follicular atresia, corpus luteum, ovarian atrophy, post-ovulatory metrorrhagia, and corpus luteum persistence. All of this leads to reduced fertility and early embryo loss at various stages of development (up to 45 days).

The current focus of veterinary gynaecology is on maintaining the health of dairy cows and ensuring high fertility. The main obstacle to addressing this issue is inflammatory and functional diseases of the reproductive organs, which manifest as prolonged infertility in cows. The diagnosis of postpartum uterine pathologies in cows is determined by clinical signs using laboratory testing methods. The main medicinal products and treatment methods are aimed at halting the inflammatory process in the uterus, restoring the microflora and improving reproductive function. In this study, methods for testing the fertility haplotypes HH2, HH6, HH7, JH1 and JH5 in Holstein and Jersey breeds, as well as their hereditary abnormalities, have been optimised and developed. Furthermore, work has been carried out on the prevention, diagnosis and treatment of gynaecological diseases in a farm setting.

The aim of the dissertation.

Improvement of genotyping methods for CD18, IFT80, SDE2, and CWC15 based on gene loci associated with embryonic mortality in cattle on farms in the Almaty region, and improving animal growth and reproductive performance through the refinement of methods for the diagnosis, prevention and treatment of gynaecological pathologies.

Research objectives:

1. To investigate the fertility haplotypes BLAD, HH2, HH6, NN7, JH1 and JH5, which are associated with embryonic mortality, on farms in the Talgar, Ili and Enbekshikazakh districts of the Almaty region;
2. Determination of the prevalence of gynaecological diseases in cows on farms in the Talgar, Enbekshikazakh and Panfilov districts;
3. Development of protocols for the diagnosis, prevention and treatment of gynaecological diseases on farms in the Talgar, Ili and Enbekshikazakh districts, and a comparative assessment of their therapeutic efficacy;
4. Identification of pathogenic microorganisms using PCR in cases of clinical and subclinical endometritis in cows.

Methods of research.

Frozen blood samples containing anticoagulants were used as research material. DNA was extracted from the biological samples using commercial kits and the conventional phenol method. The quality of the extracted DNA was assessed by horizontal electrophoresis and by measuring the DNA concentration.

The molecular genetic analysis methods included the collection of biological samples, DNA extraction, primer design, determination of amplification conditions, and genotyping of samples using horizontal PCR-PDRF electrophoresis, PCR with Tetra Primer ARMS primers, KASP, Real-time PCR, and documentation of gel analysis results.

The experimental part of the study was conducted on Holstein, Jersey and Simmental cows on farms in the Talgar and Enbekshikazakh districts of the Almaty Region and the Panfilov district of the Zhetysu Region. Gynecological examinations were carried out using the following methods: information from a specialist veterinary-gynaecological journal, reports on artificial insemination, general clinical examination, rectal examination, vaginal examination and ultrasound examination.

A metal speculum, commonly used for vaginal examinations, was employed. The vaginal speculum was used to examine the genital organs, the vagina and the cervix. In addition, attention was paid to the vaginal mucosa, its color, the presence or absence of papules, the presence or absence of pus, the appearance of the cervix, its level when closed and any abnormal discharge. During the rectal examination, the following uterine parameters were assessed: the position of the uterus, its tone, the firmness of the cervix, body and horns, the functional state of the uterus, and the presence of the ovaries.

Ultrasound examinations were performed using the Mindray Z5 Vet and PU2200 Vet scanners. These scanners were used to obtain sonograms of the uterus, ovaries and fallopian tubes, and to determine the volume and composition of the exudate accumulated in the uterine horns in cases of acute endometritis and uterine subinvolution. The endorectal probe of this device enabled us to identify the uterine branches and the nature of the exudate within them. Using the Mindray Z5 Vet B-mode linear probe with a frequency of 5–7.5 MHz, we determined the size of the ovary, the diameter of the follicles, their number and the structure of the corpus luteum, the number and size of the follicles, the thickness of the follicular walls and the echogenic stroma. The assessment of the condition was based on clinical data, the results of a rectal examination and the results of the ultrasound scan.

PCR was used to detect opportunistic pathogens in cows with gynaecological disorders. To this end, swabs were taken from the vaginal opening, the vagina and the portion of the vagina adjacent to the cervix using a sterile swab or cytology brush, and these were cultured on MacConkey agar. DNA was extracted from the colonies obtained after inoculation using commercial kits.

Scientific results, their validity and novelty.

The scientific novelty of this thesis lies in the fact that, with a view to improving methods for diagnosing genetic defects associated with embryonic loss in Holstein and Jersey cows, DNA markers were studied and the frequency of alleles of the CD18, IFT80, SDE2, CWC15, associated with embryonic loss, the prevalence of gynaecological pathologies in cows was investigated, and measures for disease prevention, diagnosis and treatment were developed.

In cows from the study farms, a rectal examination was used to determine the presence of acute endometritis, chronic endometritis and uterine subinvolution, based on signs of fluctuation in the exudate collected in the uterine cavity, the consistency of the organ and changes in the uterine topography. The ultrasound method enabled the nature of the exudate in the uterine cavity to be determined. According to the study results, treatment regimens No. 2, No. 6 and No. 10 demonstrated greater efficacy than other treatment regimens in the management of acute and chronic endometritis, uterine subinvolution, vaginitis, cervicitis and uterine atony. The duration of treatment was 5–7 days, and the recovery rate was 83.5%–92.3%. Treatment protocols No. 2, No. 6 and No. 10 proved to be effective. The comprehensive treatment protocols included medications and procedures that reduced inflammation, relieved pain, had an antihistamine effect, possessed antimicrobial properties, promoted contraction of the uterine mucosa, removed exudate and improved local blood circulation.

Conditions arising from reproductive dysfunction in cows, such as postpartum anestrus, gonadal hypofunction, ovarian follicular cysts and anovulatory oestrous cycles, result from hormonal imbalances in the body. Therefore, one method of stimulating gonadal function involves accelerating the onset of the oestrous cycle, restoring their nutritional status, and administering hormonal preparations that ensure the functional activity of the pituitary-gonadal system and regulate metabolism; as a result, we have confirmed the effectiveness of treatment using artificial insemination. The identified diseases are seasonal in nature, with dry and hot temperatures affecting fertilisation and the calving process. Furthermore, feeding with silage and dry grass, as well as the inclusion of table salt in the diet, is rare, so its quantity is small. Dry feed has a negative effect on ovarian function, causing ovarian hypofunction and atrophy. Treatment regimens No. 11 and No. 12 proved effective in treating ovarian hypofunction, follicular and luteal ovarian cysts, anestrus and persistent corpus luteum. As a result of applying these treatment regimens, affected animals were induced into oestrus, followed by artificial insemination.

During gynaecological examinations on these farms, acute and chronic endometritis were identified in cows in their second or third lactation; samples were taken from the vagina and the vaginal opening and analysed. The rapid and accurate

detection of gynaecological diseases had a positive impact on the implementation of treatment measures. The use of the PCR method proved effective for detecting microorganisms in clinical material during inflammatory processes. As a result, 33.33% of isolates from cows with chronic endometritis tested positive for *Escherichia coli*, 43.75% for acute endometritis, 62.5% and 44.44% for mixed isolates of *Escherichia coli* and *Trueperella pyogenes*, and 18.5% and 18.75% for *Staphylococcus aureus*.

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 «The creation of genetic profiles and the study of the genetics of local Kazakhstan cattle breeds using whole-genome sequencing», IRN AP19674808, (2023-2025). As part of the Ministry of Agriculture of the Republic of Kazakhstan's targeted funding programme, Research Project BR22885686: «Development of a system for the genetic improvement of beef cattle breeds using innovative methods of molecular genetics, breeding and digital technologies» (2024–2026).

Description of the Doctoral student's Contribution to Publications.

Under the supervision of academic advisors, the PhD candidate published 11 articles based on the results of their research, including 3 articles in journals published by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, and 2 articles in journals indexed in the Scopus database. Articles in the «International Journal of Veterinary Science»: title of the article «Improvement of Diagnostic Methods for Carriers of HH2 and JH1 Fertility Haplotypes in Holstein and Jersey Cows», 2025 (<https://doi.org/10.47278/journal.ijvs/2025.034>), «International Journal of Veterinary Science» title of the article «Efficiency of diagnostic methods for hypotrichosis, dilutor and osteopetrosis in local cattle breeds of the Republic of Kazakhstan», 2026 (<https://doi.org/10.47278/journal.ijvs/2025.119>). In addition, data were collected on alleles of the CD18, IFT80, SDE2 and CWC15 genes, gynaecological conditions and pathogenic microorganisms associated with embryonic loss, and these factors were taken into account in accordance with the methods employed.

Scope and Structure of the dissertation.

The thesis consists of an introduction, a literature review, a description of research materials and methods, the results of the author's own research, a discussion of the research findings, a conclusion, recommendations for industry, a list of references and appendices. The main text of the thesis comprises 134 pages, including 32 tables and 41 figures. The total length of the thesis, including the appendices, is 145 pages. The bibliography comprises 227 sources.