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STUDYING THE STRUCTURE OF VAGINAL MICROBIOMA IN HEALTHY COWS AND PURULAR - CATARAL ENDOMETRITIS COWS

Abstract

The article presents data on the study of the microbial etiology of purulent-catarrhal endometritis at Agrofirma Akas LLP using the PCR method. In dairy enterprises, cow infertility is recorded in 20-26% of the population and often leads to significant economic damage. Among the causes of cow infertility, a special place is occupied by diseases of the reproductive system. Diseases of the organs of the reproductive system are registered in 30-51% of infertile cows and in 13-28% of heifers, and the technology of keeping dairy cows contributes to the increasing role of these diseases in the etiology of infertility. The main predisposing reasons for the occurrence of low fertility are the following factors: a decrease in the body's resistance against metabolic disorders, as well as some infectious diseases that have an indirect effect through the immune status of highly productive cows or even a direct effect on fertilization, pregnancy and the postpartum period. For example, such as *Leptospirosis*, *Brucella* or *Neospora* causing abortions and, consequently, reducing milk production in herds and sometimes fertility. Studies have shown that the vagina in the microflora of healthy cows is dominated by *L. sakei*. and *W. koreensis*, while there are no dominant bacterial strains in cows with endometritis in which an increase in the number of bacteria was detected. This suggests that a violation of the microflora of the vaginal microbial community can contribute to the occurrence of endometritis.

Keywords: *purulent-catarrhal endometritis, cows, PCR, vaginal microbiome.*

Introduction. For cost-effective management in dairy cattle breeding and the timely receipt of offspring, veterinary monitoring of the fertile state of cows and heifers is required, which includes a set of measures consisting of compliance with animal health standards, a balanced diet and timely diagnosis of infertility, prevention and treatment of obstetric and gynecological pathologies. Intensive genetic selection methods have led to an increase in the milk production of cows, which leads to a decrease in fertility due to an increase in the occurrence of postpartum gynecological diseases, poor expression of estrus, and the appearance of defective oocytes, embryos, and infections of the reproductive system. Therefore, the main task of veterinary specialists is to develop a set of effective veterinary and zootechnical measures that can reduce the risks of obstetric and gynecological diseases, while maintaining milk production and increasing litter production. In dairy enterprises, cow infertility is recorded in 20-26% of the population and often leads to significant economic damage. Among the causes of cow infertility, a special place is occupied by diseases of the reproductive system. Diseases of the organs of the reproductive system are registered in 30-51% of infertile cows and in 13-28% of heifers, and the technology of keeping dairy cows contributes to the increasing role of these diseases in the etiology of infertility [1, 2].

The main predisposing reasons for the occurrence of low fertility are the following factors: a decrease in the body's resistance against metabolic disorders, as well as some infectious diseases that have an indirect effect through the immune status of highly productive cows or even a direct effect on fertilization, pregnancy and the postpartum period. For example, such as *Leptospirosis*, *Brucella* or *Neospora* causing abortions and, consequently, reducing milk production in herds and sometimes fertility. However, despite the fact that infectious diseases harm individual herds, on a larger scale, the impact of infectious diseases can be overcome with the help of specific prophylaxis [3,4].

Infection of reproductive organs in cows is one of the prevailing factors that have a negative economic impact on the dairy industry. This is one of the main factors that affects and causes infertility in the postpartum period in dairy cows. The economic damage associated with cow endometritis is about \$ 1.411 billion and \$ 650 million in the EU and the USA, respectively [5].

The uncontrolled use of antibiotics in the treatment of infectious gynecological pathologies in cows caused antibiotic resistance of microbes [6]. Therefore, it is very important to evaluate alternative methods that can be used in the treatment of infections of gynecological diseases in cows. Studies have shown that vaginal microflora are important for the health of reproductive organs and that the dominant strains of natural microflora can be used to prevent and treat vaginal infections, acting as a biological barrier or producing lactic acid, bacteriocin and hydrogen peroxide [7]. Since the vaginal microflora is important for the gynecological health of cows, a comparison of the vaginal microflora of healthy cows and patients with purulent-catarrhal endometritis can lead to the development of a potential probiotic for the prevention and treatment of endometritis in cows.

The principle of the method of polymerase chain reaction (PCR, Polymerase chain reaction, PCR) was developed by Carey Mullis (Cetus, USA) in 1983. The discovery of PCR was one of the most prominent events in the field of molecular biology over the past 20 years. For the development of PCR analysis, K. Müllis in 1993 was awarded the Nobel Prize in chemistry. The appearance of the PCR method was due to certain advances in molecular genetics, primarily the decoding of the nucleotide sequence of the genomes of a number of microorganisms. It cannot be said that PCR was made possible by the discovery of the unique taq-DNA polymerase enzyme found in bacteria that live in geysers. The peculiarity of this polymerase is its exceptional heat resistance (can withstand heating to boiling point without loss of activity) and high operating temperature (optimum operation 72 ° C).

Grace, simplicity of execution, unsurpassed indicators of sensitivity and specificity brought the method unprecedented popularity. In a short time, PCR analysis spread around the world, quickly leaving the laboratories of scientific institutes in the field of practical clinical use. Diagnosis of infectious diseases, including those caused by agents that are difficult to cultivate, genotyping of microorganisms, assessing their virulence, determining microflora resistance to antibiotics, genodiagnosics and genetic fingerprinting, prenatal diagnostics, biological control of blood products - this is not an exhaustive list of areas where successfully PCR is used.

The genome of the pathogen cell, or rather its RNA and DNA, is more conservative with respect to variability, therefore, studies are currently underway to identify the latter in pathological material.

The main stage in the recovery of animals from infectious diseases, including the gynecological pathology of cows of microbial etiology, is timely and quick diagnosis, where laboratory studies in order to detect pathogens in pathological material occupy an important place.

Materials and methods. The PCR analysis method is widely used to identify the bacterial composition of various microbial communities, such as the microbiome of the digestive and reproductive tract, since these approaches allow the detection of microorganisms that cannot be cultured on nutrient media. In connection with the above, the goal of our research was to evaluate and compare the vaginal microflora of patients with purulent-catarrhal endometritis and healthy cows using PCR and primers specific for the 16S rRNA gene (table 1) [8].

As objects of the study, vaginal swabs from 5 patients with purulent-catarrhal endometritis and 5 healthy cows of Agrofirma Akas LLP were taken.

The work was carried out on the basis of the Test Center of the Zhangir Khan West Kazakhstan Agrarian Technical University. The polymerase chain reaction was carried out on an iQ5 device from BioRad.

DNA isolation was performed by the method of CTAB. The quality of DNA extraction was determined using agarose gel electrophoresis.

We carried out gynecological examinations of 42 (goal) cows owned by Agrofirma Akas LLP in the rural district of Pogromny, Terektinsky district of the West Kazakhstan region of the Republic of Kazakhstan, took into account the following categories of breeding stock: pregnant females to be examined for pregnancy (1.5-3 months after insemination), infertile.

Table 1 - Bacterial 16S-oriented primers and annealing temperature for PCR

Bacteria (amplicon size)	Oligonucleotide sequence (5'-3')	Annealing temperature (C°)
<i>Clostridium perfringens</i> (212 bp)	F: ATGATTGGGATTATGCAGCAA R: TCCATCCTTTGTTTTGATTCCA	56
<i>Fusobacterium spp.</i> (273 bp)	F: C(A/T)AACGCGATAAGTAATC R: TGGTAACATACGA(A/T)AGGG	54
<i>Enterococcus spp.</i> (144 bp)	F: CCCTTATTGTTAGTTGCCATATT R: ACTCGTTGTACTIONTCCCATTGT	61
<i>Prevotella spp.</i> (179 bp)	F: GGGATGCGTCTGATTAGCTTGTT R: CTGCACGCTACTTGGCTGGTTC	62
<i>E. coli</i> (340 bp)	F: GTTAATACCTTTGCTCATTGA R: ACCAGGGTATCTAATCCTGTT	60
<i>Weissella spp.</i> (725 bp)	F: CGTGGGAAACCTACCTCTTA R: CCCTCAAACATCTAGCAC	61
<i>Bacteroides spp.</i> (140 bp)	F: AAGGGAGCGTAGATGGATGTTTA R: CGAGCCTCAATGTCAGTTGC	59
<i>Lactobacillus spp.</i> (186 bp)	F: CGATGAGTGCTAGGTGTTGGA R: CAAGATGTCAAGACCTGGTAAG	58

Results of study. In vaginal studies, it was noted that the mucous membrane of the vagina is pink in color, there was a slight hyperemia, edema. In the vaginal cavity, the accumulation of exudate is a mucous membrane of a whitish color with a liquid consistency.

During rectal examination in non-pregnant cows, the uterus is located longitudinally in the middle part of the pelvic cavity and the cervix is felt. The presence of fluid in the uterine cavity, asymmetry, an increase in the uterine horns, swelling, the absence of contractile function of the walls of the uterus and the presence of a persistent corpus luteum in the ovaries were also observed. When examining cows with pathologies of the reproductive organs, a decrease in the contractility of the walls of the uterus was noted. Among the most common diseases in the studied cows, endometritis, ovarian cysts and a persistent corpus luteum are recorded, less commonly cervical induction, uterine atony and hypotension

During the study, the following physiological state of the cows was established (table 2).

Table 2 - the Physiological condition of cows LLP AF «Akas» (n = 42)

Physiological groups (%)		
pregnant	barren	without pathology
20 (48,0%)	21(50,0%)	1(2,0%)

From the data of table 1, it is seen that in 50.0% of the studied cows, genital pathology was revealed (endometritis, cervical induction, atony and hypotension of the uterus, follicular cysts and persistent corpus luteum). Infertile cows 21 (50%) underwent a thorough gynecological examination to identify various gynecological diseases of the cows, which are indicated in the graphic drawing 1.

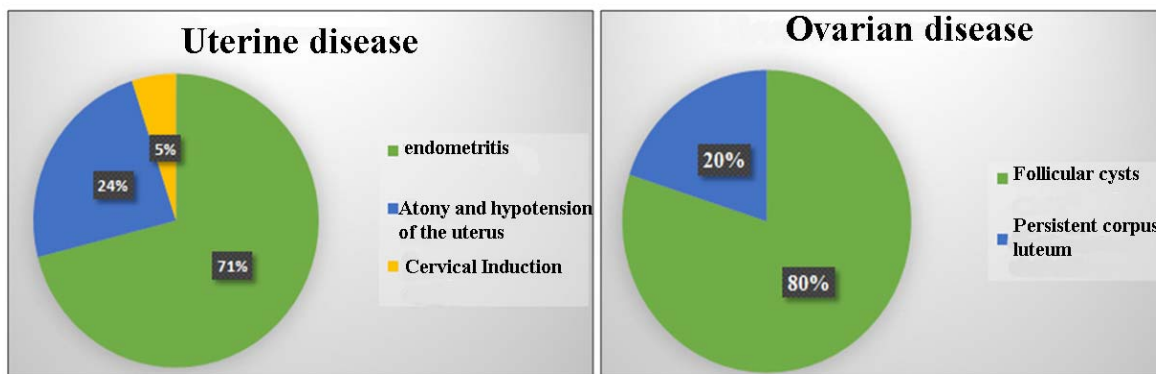


Figure 1 - Pathologies of the reproductive system in infertile cows of AF Agas LLP (n = 21)

From graphic figure 1, it can be seen that of the studied cows of uterine disease, endometritis is often found in 71% of cases, and less often cervical induction in 5% of cases. Follicular cysts are often reported from ovarian disease in 80% of cases.

During molecular biological studies, the vaginal microflora of patients with purulent-catarrhal endometritis and healthy cows was evaluated and compared using PCR and primers specific for the 16S rRNA gene (figure 2).

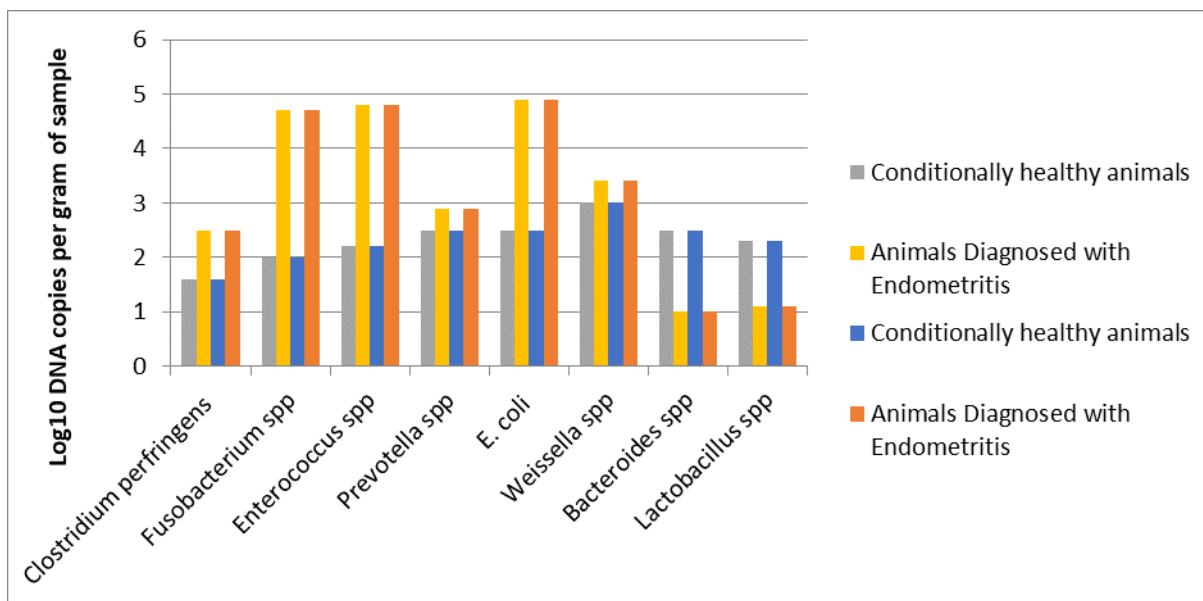


Figure 2 - microflora in relatively healthy cows and cows with endometritis

The analysis showed that *Lactobacillus spp.*, *Pediococcus spp.*, *Leuconostoc spp.*, *Weissella spp.*, *Enterobacteriaceae*, *E. coli* and *bacilli* were present in the vaginal mucus in relatively healthy cows and cows with endometritis. The results of studies obtained by PCR correspond to the clinical manifestations of endometritis, in which the natural microflora is disturbed and the number of pathogenic bacteria increases. One of important factor in the occurrence of endometritis is that bacteria from the vagina contaminate the uterus. The amount of *E. coli* and *Fusobacterium spp.* significantly exceeds in cows with endometritis, which indicates a mixed infection. In addition, microorganisms such as *B. fragilis*, *P. dentalis*, *C. perfringens* and *E. coli* are common in the vagina of healthy and cows with endometritis, confirming previous observations.

Conclusion. Studies have shown that the vagina in the microflora of healthy cows is dominated by *L. sakei*. and *W. koreensis*, while there are no dominant bacterial strains in cows with endometritis in which an increase in the number of bacteria was detected. This suggests that a violation of the microflora of the vaginal microbial community can contribute to the occurrence of endometritis.

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ТҮЙІН

Мақалада ПТР әдісін қолдана отырып, «Агрофирма Акас» ЖШС-де іріңді-қатаральды эндометриттің микробиологиялық этиологиясын зерттеу туралы мәліметтер келтірілген. Сүт бағытындағы кәсіпорындарындағы сиырлардың бедеулігі 20-26% - тіркеледі және көпшілік жағдайда экономикалық шығындарға әкеледі. Сиырлардың репродуктивті органдарының аурулары 30-51% тіркелсе, сиырлар мен қашарлардың бедеулігі 13-28% құрайды, сүтті бағытындағы сиырларды ұстау технологиясында бедеуліктің этиологиясы осы аурулардың өршуіне себепші болып табылады. Негізгі төменгі ұрықтандыру ға себепші көрсеткіштер болып келесідей факторларды атаймыз: организмнің метаболизм бұзылуынан резистенттілігінің төмендеуі, сонымен қатар жоғары өнімді сиырлардың иммундық жүйесіне жанама әсер етуі немесе буаздық және туудан кейінгі кезеңге тікелей әсер ететін кейбір жұқпалы аурулар. Мысалы, түсік тастауға әсер ететін жұқпалы аурулар лептоспироз, бруцелла немесе неоспора, сонымен қатар сүт өнімділігін кей жағдайда төлдеудің төмегі нәтиже алуына себепші болып табылады. Зерттеулер көрсеткендей, сау сиырлардың қынаптық микрофлорасында *L. sakei* и *W. koreensis*, басым болатыны көрсетілген. алайда эндометрит белгілері бар сиырларда бактериялардың басым штамдары көрсетілмеген, Жатыр микрофлорасының өзгеруі эндометрит ауруларының бірден бір белгілеріне себепші болатыны айқындалған.

РЕЗЮМЕ

В статье приведены данные по изучению микробной этиологии гнойно-катарального эндометрита в ТОО «Агрофирме Акас» с применением метода ПЦР. В условиях молочных предприятиях бесплодие коров регистрируется у 20-26% поголовья и часто приводит к значительному экономическому ущербу. Среди причин бесплодия коров особое место занимают заболевания половой системы. Болезни органов половой системы регистрируются у 30-51% бесплодных коров и у 13-28% телок, а технология содержания молочных коров способствует возрастанию роли этих заболеваний в этиологии бесплодия. Основными предрасполагающими причинами для возникновения низкой оплодотворяемости являются следующие факторы: снижение резистентности организма на фоне нарушения метаболизма, а также некоторые инфекционные заболевания оказывающие косвенное воздействие посредством иммунного статуса у высокопродуктивных коров или даже прямого воздействия на оплодотворение, течение беременности и послеродового периода. Например такие как *Leptospirosis*, *Brucella* или *Neospora* вызывающие аборт и, следовательно, уменьшающие выработку молока у стад и иногда плодовитость. Исследования показали, что влагиалище в микрофлоре здоровых коров преобладает *L. sakei* и *W. koreensis*, при этом нет доминирующих бактериальных штаммов у коров с эндометритом, у которых было обнаружено увеличение количества бактерий. Это говорит о том, что нарушение микрофлоры влагиалищного микробного сообщества может способствовать возникновению эндометрита.

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EVALUATION OF POLYMORPHIC VARIANTS OF GENES OF SOMATOTROPIN CASCADE AS GENETIC MARKERS OF MEAT PRODUCTIVITY IN DOMESTIC CATTLE BREED

Abstract

It is known that growth hormone (GH-somatotropin) is the most important growth regulator in mammals. Synthesis of somatotropin and the realization of its physiological effects is a chain of successive interactions of the protein receptor (self-tropic cascade). The key links of this chain are the pituitary transcription factor-1 (bPit- 1), triggering the expression of the genes of somatotropin and prolactin, prolactin and growth hormone, regulating lactation, the growth hormone receptor (bGHR), which transmits the somatotropin humoral signal to target cells. Thus, based on the researches performed, the following data is stated below. On the first hand, specified allele frequencies for all the polymorphisms in question are comparable with the other authors' data; rare allele of other breed representatives are also rare according to the results of our research. The research works were performed within the frameworks of the scientific project of the Ministry of Education and Science of the Republic of Kazakhstan.

Keywords: DNA, somatropine cascade, polymorphism, gene, cattle.

Introduction. Nowadays, the need in modernization of beef breeding in the Republic of Kazakhstan has been arisen. In order to solve this problem, strengthening of selection of local breed is needed together with delivery of highly-productive breeds of foreign selection. This means Auliekol bovine cattle breed having stable immunity to the diseases spread on the territory of the Republic of