A study on modeling infectious metritis in dairy cows

Yvan Kalyuzhny[1], *Rosa* Yavlieva[2], *Zoya* Morozova[3], *Grigory* Firsov[3*], and *Oleg* Budtuev[3]

[1]Federal State Educational Institution of Higher Education Saratov State University of Genetics, Biotechnology and Engineering named after N.I. Vavilov, Saratov, Russia
[2]Sechenov University (Sechenov First Moscow State Medical University), 6-1, Bolshaya Pirogovskaya St., Moscow, 119991, Russia
[3]Federal State Educational Institution of Higher Education, Volgograd State Agricultural University, Volgograd, Russia

Abstract. The study highlights the work on the creation of a method for assessing reproductive capacity in metritis of dairy cows on the model of laboratory rodents in experimental conditions, as well as the study of the effect of material of uterine secretions of cows on the functioning of the reproductive system of female rats after childbirth. The article presents the methods of reproduction of infectious metritis of dairy cows of Holstein-Friesian and Simmental breeds on female Sprague Dawley rats. The method of infecting uterine tissues of laboratory rats with microbial cocktail obtained from Holstein and Simmental cows with postpartum metritis is proposed. Availability of the procedure of intravaginal inoculation, well-defined localization of the effect without systemic spread of the infectious process and significant results of infection advantageously distinguish this model from other studies. The effect of the mixture of microorganisms used for infection on rat uterine tissues was studied. The results of measuring rectal temperature and body weight indices of laboratory rats after modeling of infectious metritis are presented. A statistically significant correlation between the results of rectal temperature and body weight was established. The significance of Mann-Whitney U-criterion and Wilcoxon T-criterion was revealed.

1 Introduction

An inflammatory disease caused by bacterial invasion of the uterine tissue resulting in pus formation, detected three weeks after calf birth, is defined as clinical metritis. [1-3] This pathology occurs in 15 to 20% of cows during the lactation period and is a serious problem because even after the symptoms disappear, the animals experience a delay in fertilization. [4-5] As a result, they are more often candidates for herd exclusion due to reproductive problems compared to healthy individuals without metritis. Establishing a pilot animal clinical study design will provide insight into how uterine abnormalities affect reproduction and reproductive function. The most common causes of infectious diseases in cows are

* Corresponding author: firsovgm@yandex.ru

Gram-negative microorganisms such as *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum* and *Staphylococcus aureus*, which are the main causative agents of these conditions. [5-6] The bacterial toxin that provokes uterine inflammation after childbirth affects both the reproductive system directly and the body as a whole, affecting the ability to conceive. The inflammatory process leads to disruptions in the synthesis of prostaglandins PGF2α and PGE2 in the uterine mucosa. [7] This results in a shift in immune mediators and cytokine production, which can affect the functioning of glands such as the hypothalamus and pituitary glands. The use of animal models has both certain disadvantages and advantages. Failure to use such models, including transgenic and mice with genetically modified lack of certain genes, would significantly narrow progress in the study of infectious diseases and the fundamentals of reproductive biology. Although no model can completely match pathologies in cows, analogies between genital infections in humans and rodents (presence of inflammatory cytokines in amniotic fluid, placental damage, and characteristic signs of negative pregnancy outcome) support the feasibility of using rodent models [6-9]. Laboratory models of uterine infections using rodents (mice and rats) as subjects have been proposed for ruminant diseases such as brucellosis, campylobacteriosis, and trichomonosis [6-9]. Advantages of using rodent-based models include their relatively rapid reproduction, the high productivity of a single female in terms of offspring, and the availability of the necessary reagents for these animals. Finally, the use of a rodent-based model is significantly less expensive compared to the use of a bovine model, making it an economically more attractive option.

The aim of this scientific study was to create a methodology for experimental evaluation of metritis in experimental rodents and to analyze the effect of combined treatment regimens of this disease on the state of reproductive organs of female rats after delivery..

2 Materials and methods

2.1 Animals

Female Sprague Dawley rats weighing 220-240 grams were subjected to experimental infection after completion of natural births. Rats were kept in laboratory cages SR-6 (ProfLab, RU) individually on sterilized sawdust bedding. Feeding was carried out with a standard feed pelleted ration, drinking from nipple auto-drinkers in full. Two groups of animals totaling thirty animals were taken for the experiment, n=10 1st experimental group infected with material from Holstein-Friesian cows, n=10 2nd experimental group infected with material from Holstein-Friesian cows and n=10 control group consisting of intact females.

An opinion of the Biomedical Ethics Committee on the compliance of the experiments with the principles of humane treatment of animals was obtained.

2.2 Experimental design

In 2024, intrauterine environment samples were obtained from ten female Holstein-Friesian cattle that had a history of calf birth (two to three times). These animals were kept in open paddock conditions and had straw-covered stalls. On average, each gave 9124 kilograms of milk during the lactation period. The diet included green fodder, corn silage and hay enriched with mineral components. All animals belonged to SP Donskoe LLC, located in Kalachevsky District.

Also in 2024, intrauterine environment samples were obtained from five female Simmental cattle that had a history of calf birth (two to three times). These animals were

free-range and had straw-covered stalls. On average, each gave 8108 kilograms of milk during the lactation period. The diet included green fodder, corn silage and hay enriched with mineral components. All animals belonged to the farm "Zubkov A.A." located in Sredneakhtubinsk district.

Before taking samples from the uterus of each cow, the vulva was cleaned using paper napkins for single use, followed by treatment with 70% ethanol solution. To prevent possible contamination during the sampling process, the sampling instrument was secured using a disposable plastic catheter and a protective plastic sheath. In order to increase the biomass of microorganisms, they were further cultured in a nutrient medium based on meat-peptone at 37 degrees Celsius for 24 hours.

To provide anesthesia in rodents of both experimental groups, intramuscular injection using the drug telazol® (combination of tiletamine hydrochloride and zolazepam hydrochloride) at a dose of 20 mg per kilogram of weight was applied, after which they were fixed in the dorsal position. The procedure of intravaginal administration of the microbial mix was performed using a MicroPette Plus 10-100 μL automatic pipette, (DLab, China). For infection, a suspension suspension of bacteria cultured from sample material from Holstein-Friesian cows for the 1st experimental group and a suspension of bacteria cultured from sample material from Simmental cows for the 2nd experimental group were used, which was dosed to a volume of 25 μL and a concentration of $1x10^6$ colony-forming units per rat. The bacterial population level was measured according to McFarland's standards. A microbial suspension of 0.5 McFarland density was prepared under aseptic conditions and then diluted with sterile 0.89% NaCl solution until the required concentration was reached. The pipette was adjusted to accurately measure the indicated amount of suspension. With care, the pipette tip was carefully inserted into the vaginal lumen and advanced through the cervix into the uterine space, while carefully controlling the position of the instrument to avoid entering the urinary tract of the rat.

The procedure of intravaginal injection of bacterial suspension material was carried out through the cervical canal using a suspension obtained by tissue culture from the uterus of dairy cows (n=15), which five days after calving were diagnosed with postpartum inflammatory process in the uterus. In order to create an experimental modeling of metritis in cattle, intravaginal administration of bacterial suspension after natural delivery of female rodents was used.

Daily monitoring during the two weeks of the experiment included observation of behavioral responses, health status, respiratory functions, excretions, stools, feeding and water consumption in each individual. The animals' weight and rectal body temperature were recorded as part of the study. In addition, blood was drawn from the tail vein for standard laboratory tests. During the recovery period after manipulation, the rodents were kept in an environment where they had unrestricted access to food and fluids.

2.3 Statistical analysis

To perform statistical analysis, the specialized statistical program IBM SPSS Statistics version 22, developed by IBM Corp. in the United States of America (IBM Corp., USA), was used.

In the course of calculations, the Mann-Whitney U-criterion for comparison of two independent sample groups and the Wilcoxon T-criterion for two dependent samples were applied. Data were reported in the form of mean with standard error (±SD), with the significance level set at less than 0.05.

3 Results and discussion

The use of laboratory specimens is key to analyzing bovine diseases, as much of the research in veterinary medicine relies on approaches that are not economically feasible to apply to animals intended for production [1-3]. It is possible to introduce pathogens into the pelvic cavity region using sponges impregnated with bacterial culture within the vaginal space. However, the protective role of the cervical canal makes it difficult for microorganisms from the vaginal microbiome to penetrate the upper reproductive system, which usually remains sterile. However, many proposals have been made for the use of combined infections to model metritis in laboratory animals that include internal pathogens, such as anaerobes, which may be of key importance in the development of uterine inflammation in cows [6].

The rat uterus is small in size, which makes it difficult to perform surgical interventions and analyze experimental data. At the same time, the uterus of this rodent is characterized by special histological features and occupies a significant part of the pelvic genital area. That is why the model of inflammatory uterine diseases on rats is often used for research [6]. The main cause of uterine inflammation in cows is often infection, which is provoked by various bacteria (both aerobes and anaerobes), mycoplasmas, chlamydia or viruses. In order to more accurately recreate the mechanism of uterine inflammation in cows, scientific work is increasingly using gradual introduction of pathogens to create laboratory models of these conditions. Inflammatory processes in the body often result from the introduction of pathogens into uterine tissues during acute infectious conditions.

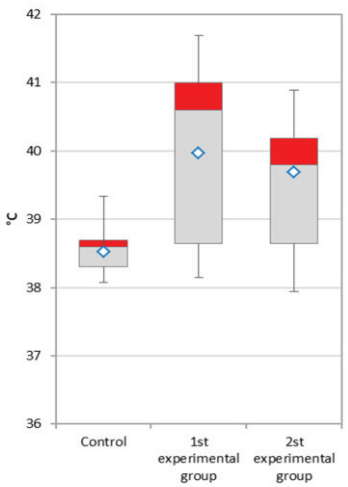

Fig. 1. Box plot of changes in rectal temperature of rats in the control and experimental groups.

In rodent experiments, rectal temperature was used to determine signs of acute inflammation of the uterus, which served as an indicator of changes in the body temperature regime of animals Clinical symptoms, including an increase in rectal temperature, became noticeable after two days from the moment of infection. On the second day following the intrauterine injection of infectious material, an increase in temperature was observed in both the first and second control groups by ±SD 2.62±0.82 and 2.70±0.77 °C, respectively. In the period from the fourth to the tenth day, rectal temperature in animals from the first group significantly exceeded the same indicators in the second group by 1.4-1.6 °C, which indicates the spread of the inflammatory process in the zone of pelvic organs to the whole

body of experimental rats after infection and caused an increase in body temperature, as well as a systemic inflammatory reaction (Figure 1).

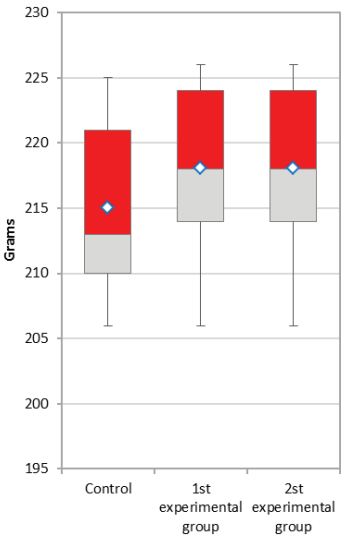

Fig. 2. Box plot of changes in body weight indicators of rats in the control and experimental groups.

Analyzing the data on rectal temperatures collected during a two-week experiment involving the first experimental and control groups of rats revealed that there were no significant differences between them according to the results of the Mann-Whitney U-criterion. Performing analysis of rectal temperature data collected during the two-week experiment involving the second experimental and control groups of rats revealed that there were no significant differences between them according to the results of Mann-Whitney U-criterion. Applying Wilcoxon's T-criterion to the conjugated groups within the experiment with rats from the first experimental group, it was found that the differences in performance between the third and fourteenth days of the study were statistically significant at an error confidence interval of 5%. The asymptotic two-sided p-value was 0.04.

In the first experimental group, there was a decrease in mean ±SD weight from 227±9.23 to 205±7.65 grams in the first forty-eight hours after infection. The second group of subjects showed a similar decrease in body weight indices: from 231±8.44 to 210±6.62 grams during the same time period (Figure 2). Analysis of the data on the change in body weight indices collected during the two-week experiment involving the first experimental and control groups of rats revealed no significant differences between them according to the results of the Mann-Whitney U-criterion. Conducting an analysis of the data on the change in body weight indices collected during a two-week experiment involving the second experimental and control groups of rats revealed that there were no significant differences between them according to the results of the Mann-Whitney U-criterion.

When statistical analysis was applied to the data on body weight fluctuations in rodents from the second experimental group, the use of Wilcoxon's T-criterion to compare related samples revealed significant differences in performance on the third day of the study and on the fourteenth day. These changes were statistically significant with an error confidence interval of 0.01. The asymptotic two-sided p-value was 0.01.

This paper utilizes the intrauterine infection method developed from the studies of D.W. Agnew et al. (2008) [9], L. Fan et al. (2021) [7], M. Makav et al. (2022) [6] and J. Xi et al. (2023) [8]. This method involves direct intrauterine injection procedure to introduce

pathogens. The results of the studies performed on rectal temperature change and body weight change gave values comparable to the results obtained by 2021 L. Fan et al. [7]

4 Conclusion

This model is characterized by a number of advantages: ease of intravaginal injection, lack of systemic complications and a high probability of infection. The simplicity of the vaginal injection technique, well-defined local effects, lack of systemic diffusion, and superior infectivity rates make this model exceptional compared to other experiments.

The use of a laboratory model of bovine metritis in rodents can greatly simplify the study of the mechanisms of this disease, since experiments on the natural host require considerable time and expense.

This study clearly highlighted the features and stages of development of induced inflammation in the uterine region in laboratory rodents. The comprehensive arguments have contributed to the knowledge of the mechanisms of onset and development of infectious metritis, which may be useful for further studies on the management of this disease in cattle.

The rodents who participated in the pilot phase of the study experienced an acute stage of inflammation during the first two weeks, accompanied by an increase in their basal temperature and loss of body weight.

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