Preclinical examination of complex antimicrobial preparation for treating endometritis in cows

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Abstract. According to a statistical study an average of 40 % of infertile animals have obstetric and gynecological pathologies. Among obstetric and gynecological pathologies, the most common is an acute postpartum endometritis, which make up from 37.8 to 41 %. Therapy and Pharmacology department of the Kuban State Agrarian University has developed a drug for the cows postpartum endometritis treatment. Conducted preclinical tests prove its harmlessness, as a result of which it belongs to hazard class 4. It should be noted that the biochemical blood parameters of rats in the experiment do not go beyond the reference values. The drug has a pronounced antimicrobial effect. MBC is from 7.8 mg to 31.2 mg while MIC is from 31.2 to 62.5 mg for such strains as S. aureus, E. coli, St. xylosus, Ps. aeruginosa, P. vulgaris. The developed drug does not have an irritating and sensitizing effect and exhibits a regenerative effect. Conducted experiments on the wound healing effect of the drug prove that this drug has a high regenerative effect. The average wound healing area was 20.4 % per day.

1 Introduction

"The main reasons that reduce the broodstock reproductive rate are postpartum functional and inflammatory diseases of the cattle genital organs that cause symptomatic infertility." [1]. According to the research of I. Konopeltsev [2], E. P. Agrinsky, [3] "uterine diseases after labor do not tend to decrease, despite the successes achieved in the diagnosis, prevention and treatment of highly productive dairy cows".

The causes of gynecological pathology are diverse and in most cases are in complex action. Such reasons include detention conditions violation, lack of active exercise, unsatisfactory microclimate of livestock farms.

Birth and postpartum complications lead to the uterus inflammatory processes. In the first week after labor, endometritis, as a rule, develops against the background of delayed placenta or acute uterine subinvolution [4, 5].

One of the factors leading to purulent inflammatory diseases and weakening of the uterus contractile function in cows is an increased level of stable metabolites of nitric oxide [6].

The combination of these and other factors leads to the occurrence of postpartum endometritis in cows, which in turn is one of the main reasons for the dairy cattle reproductive health decrease.

The ongoing therapeutic and preventive measures do not always allow achieving the expected effect, since endometritis of bacterial and mycotic etiology is quite common at present, as evidenced by the data of a number of authors who claim that sick cows cervical mucus microbiological examination shows its high contamination by pathogenic and conditionally pathogenic microorganisms [7]. Moreover, an important property of modern drugs aimed at the treatment of postpartum endometritis is the ability to exhibit a wide range of pharmacological activity and being harmless.

2 Results and discussion

Over the past 5 years, 41,403 cows were rejected on the farms of the Krasnodar Territory due to irreversible infertility. In 2018, 9,491 animals were sent to slaughter, which is 7.8 % of the number of cows at the beginning of 2018.

According to the Krasnodar Territory Veterinary Medicine Department reports over the past 5 years infertile animals accounted for about 17 % as of January 1 of the new year, of which an average of 60.1 % were animals with a normal genital device.

Thus, annually on average 40 % of infertile animals have obstetric and gynecological pathologies. According to the same statistics (Fig. 1), the most common among obstetric and gynecological pathologies are acute postpartum endometritis, which ranges from 37.8 to 41 %.

Functional disorders of the ovaries, in the form of hypofunction, cysts, persistent corpus luteum, also occupy a large percentage. Pathology data accounts for 18 to 24 % of all gynecological diseases. Another widespread birth disease is retention of the placenta,

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which accounts for from 16 to 18 %. It was noted that a large percentage of acute postpartum endometritis is observed in animals with severe labor and retention of the placenta.

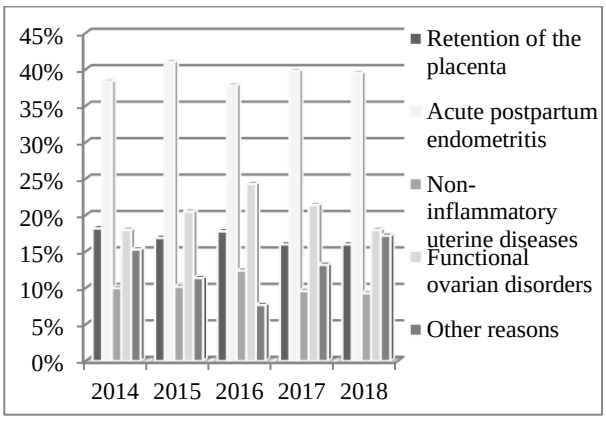

Fig. 1. Spread of gynecological pathology in the farms of the Krasnodar Territory

Thus, the development and creation of new pharmacological drugs aimed at treating endometritis of cows is an urgent area.

At the Kuban Agrarian University Therapy and Pharmacology Department a complex local preparation for the postpartum endometritis treatment has been developed.

Laboratory studies of the drug antimicrobial activity (Figure 2) indicate that the drug is in relation to S. aureus, St. xylosus and Ps. aeruginosa possesses 7.8 mg MBC, and 15.6 and 31.2 mg for E. coli and P. vulgaris, respectively. At the same time, MIC of the tested drug in relation to S. aureus and E. coli, St. xylosus is 31.2 mg, and to P. vulgaris – 62.5 mg.

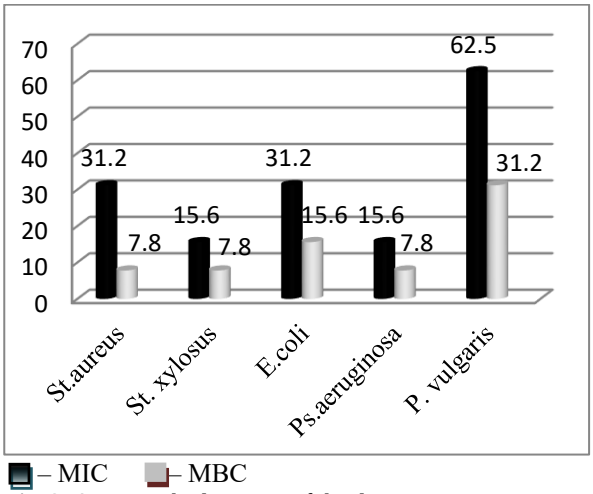

Fig. 2. Antimicrobial activity of the drug

Thus, the research results indicate that the developed drug has a high antimicrobial activity and a wide spectrum of action, which indicates the feasibility of its use for the postpartum diseases treatment in cows.

Assessment of acute toxicity was performed by the oral route of administration.

The drug was administered to experimental animals by a single intragastric administration using a syringe and probe. The concentration of active substances in 1 ml was according to the recipe. In rats of the first group, the drug was administered intragastrically at a dose of 8 ml. Rats of the second group served as control, animals of this group were injected with 0.9% sodium chloride solution, in the volume of the test drug administered to the animals.

To determine subchronic toxicity, animals were selected according to the principle of paired analogues, which were kept under identical conditions of feeding and keeping. The experiment involved 20 sexually mature outbred white rats weighing 150–180 g. The rats of the first group (n = 10) were given the test drug in free access instead of water for 14 days. The second group of animals (n = 10) served as a control.

The animals were closely monitored, given their behavior, general condition and appetite.

As a result of the determination of acute toxicity, toxic phenomena and death in laboratory animals were not observed for the entire observation period.

When studying subchronic toxicity, we also did not observe any changes in behavior, general condition and appetite. Animals behaved in the same way as animals from the second control group. Throughout the duration of the experiment, no deaths were observed.

A study of the body weight parameters and internal organs of rats in the study of subchronic toxicity showed that repeated per use did not negatively affect the clinical homeostasis of rats. Over the course of the experiment, when the drug was used for 14 days and after a 10-day recovery period, no deaths were observed in all experimental groups.

The results of gain measuring of white rats during the experiment are presented in Fig. 3.

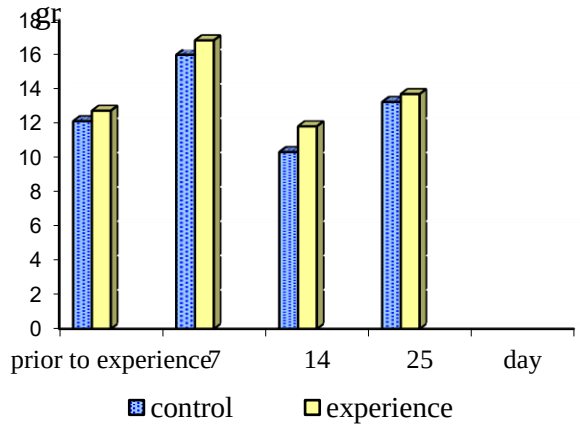

Fig. 3. Dynamics of weight gain of white rats with the introduction of floropene

As it follows from the data presented in table 1, the test drug per administration for 14 days to white rats in the studied doses does not have a statistically significant effect on the relative mass of the internal organs throughout the experiment.

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Table 1. Internal organs mass changes results coefficient of rats (M±m)

Organ name	Testing drug	Bound
After prolonged administration		
Hepar	31.3±1.52	31.1±1.61
Renes	4.38±0.67	4.52±0.36
Pulmo	5.01±0,26	5.48±0.35
Lien	4.00±0.11	3.98±0.35
Cardis	4.14±0.20	3.95±0.25
After a 25 days recovery period		
Hepar	32.06±1.34	32.3±0.26
Renes	5.19±0,47	4.90±0,41
Pulmo	5.39±0.53	5.57±0.41
Lien	4.13±0.21	4,17±0.32
Cardis	4.32±0.17	3.97±0.27

During the experimental group rats' internal organs anat-pathological study, no changes in their structure were observed.

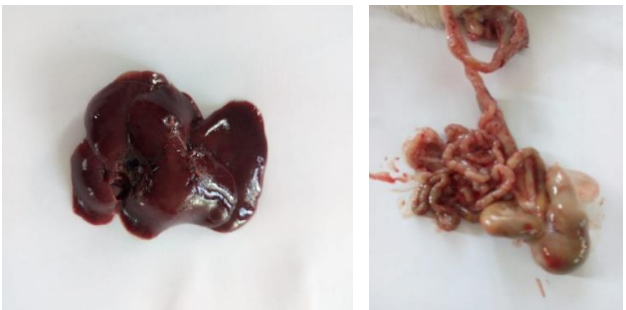
The mucous membrane of the mouth, nose and eyes is shiny, pale pink. The skin of the auricles is pale pink. The skin is elastic, pale pink. Subcutaneous tissue is well developed, pale pink. Skeletal muscles are blood-filled, pink, moderate humidity, well developed. When opening the chest and abdominal cavities, the location of the internal organs was anatomically correct. The serous membranes of the cavities had a bright pink color, a smooth and shiny surface.

The mucous larynx is pale pink, folded, shiny. The mucous membrane of the esophagus is smooth, shiny, pale pink. The mucous membrane of the stomach and intestines is gray-pink in color without symptoms and hemorrhages. The capsule of the kidney was easily removed; the cerebral and cortical substance is clearly distinguishable in the section.

The submandibular lymph nodes are pale pink, smooth, shiny, elastic consistency. Tracheal rings are white.

The heart is rounded, elastic consistency. Myocardium in a section of bright red color moderates density. The pericardium and epicardium are smooth, shiny. Heart valves are thin, smooth and shiny.

Clearance of the trachea and bronchi is free. Lungs of usual form are with a pinkish-red surface, smooth, shiny, elastic consistency. The section shows lobation, lung tissue of a pale pink color (fig. 4).



Experimental group rat liver — The stomach and intestines of the experimental group rat

Fig. 4. Rats internal organs pathological picture

The biochemical blood parameters studies results of experimental rats are shown in table 2.

Table 2. Biochemical blood parameters of experimental animals

Indicator	Bound	Experimental	Control
ALT	110.0–140.0	109.5±0.23	92.1±1.28
AST	72.0–196.,0	106.6±0.14	77.4±0.21
ALP	69.0–76.0	87,9±0,21	96.8±1.32
CRNN	17.68–70.72	54.9±1.65	66.7±0.55
TP	69.0–76.0	82.4±1.65	84.5±1.23
Glucose	2.77–7.49	7.2±0.12	7.6±0.11
Cholesterin	1.03–3.36	1.3±0.15	1.5±0.10
Albuminum	26.0–35.0	31.6±1.23	38.3±1.68
Urea	3.5–6.7	5.4±1.58	7.6±1.54
Bilirubin	3.24–10.94	12.7±1.54	18.1±1.46

Therefore, the developed drug for the degree of impact on the body of warm-blooded animals refers to low-hazard substances.

As a result of a study of sensitizing and irritating effects, the following results were obtained: instillation of the drug into the lower conjunctival sac does not cause conjunctival reddening after administration, there was no clouding of the cornea of the eye and the iris was without any visible changes, and hematosis (conjunctival edema) and discharge from eye.

The day before the study, the hair was cut in areas of approximately 10×15 cm on both sides of the back for application and observation.

Apply 0.5 ml of the test drug to the skin and fix it with a bandage.

Monitoring the reaction of the skin is carried out in natural or close to natural artificial light. With a single exposure, the state of each skin area where the applications were performed was recorded 1, 24, 48 and 72 hours after removal of the samples.

In the second series of experiments, the irritating effect was determined by the method of cutaneous applications. During the study, the formation of erythema and skin edema was not observed in experimental animals, as a result of which the index of primary irritation is zero.

The study of the drug sensitizing effect by the method of maximum sensitizing effect. The study was conducted on guinea pigs. The animals were monitored, noting the presence of edema, erythema, etc. on the skin in the area of applications with the drug. We noted that 24, 48, and 72 hours after the provocative test and removal of the dressing, positive skin reactions (edema, erythema, bladder) were not detected.

Thus, the drug does not have irritating and sensitizing effects on tissues in the area of its use.

We also conducted experiments to determine the wound healing effect of the drug.

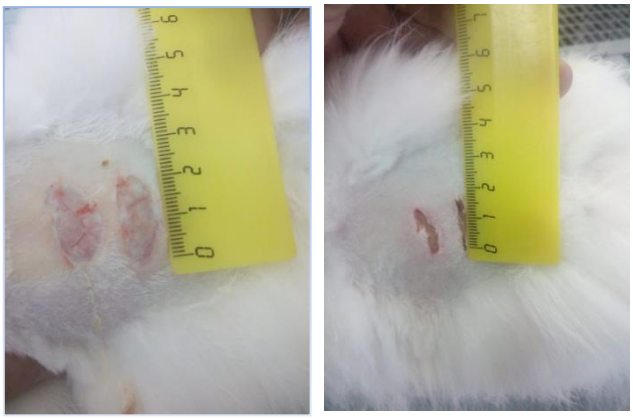
The essence of the method is to determine the nature and speed of wound healing by recording the wound surface area rate of decrease.

For the experiment, laboratory rabbits were used.

The day before the studies, the hair was cut in areas of approximately 10×15 cm. The prepared area was treated with 70° ethanol, local anesthesia was performed

with 2 % novocaine solution, 2–3 linear cuts were made using a stencil, spindle-shaped, 40×15 mm and 1.5 to 20 mm deep. The distance between the cuts was not less than 5 cm. The first cut (left) was treated with an experimental preparation, the second with a control.

As a result of the experiment, it was found that this tool has a high regenerative effect. The average wound healing area was 20.4 % per day (Fig. 5).



The first day after wounding | The third day of wound healing

Fig. 5. First and third days after the application of experimental wounds

During the embryotoxic action of the test drug, the test drug was administered to female white rats per os. Since the studied drug will be used intrauterine to cows only in the postpartum period no more than 5–7 times, it was administered from 3 to 6, from 9 to 12, and from 16 to 19 days of pregnancy at a dose of 5 ml per 1 rat (n = 10) to assess embryotoxicity) The control group (n = 10) did not administer the drug. The state and behavior of pregnant animals were monitored throughout the experiment. On the 20th day of pregnancy, 5 females of the experimental and control groups were euthanized and an autopsy was performed. The number of yellow bodies, living and dead fetus was counted.

As a result, it was found that all animals were pregnant without pathological changes, which was reflected in the behavior, consumption of food and water, as well as the condition of the hairline and mucous membranes. The body mass dynamics of white rats, which were administered the experimental drug and animals of the control group, was positive and did not have significantly significant differences.

The test drug in a therapeutic dose does not affect the fecundity of rats.

The number of corpus luteum and implantation sites per female on average in groups is practically the same. No significant differences were found in terms of pre- and post-implantation deaths. However, the total embryonic mortality in rats of the experimental groups was slightly lower than in the control group.

An external fetus examination under a microscope did not reveal the presence of any abnormalities or delayed fetal development in experimental animals. The size and weight of the rat pups of the experimental and control groups did not have significant differences.

Testing the dose was carried out on cows of patients with acute purulent-cataral endometritis. For this, 2 groups were created (n = 20). To the cows of the first group, the drug was administered intrauterine at a dose of 50 ml. To the cows of the second group, the drug was used in a dose of 100 ml.

The drug was used every other day until a therapeutic effect is obtained (Table 3).

Table 3. Development of a therapeutic dose of the preparation

Dose of preparation	Therapeutic effect		Number of days of preparation use
	cows	%	
Dose of preparation 50 ml	4	40	7
Dose of preparation100 ml	7	70	5

As a result, the following data were obtained. In the first group, the therapeutic effect was 40 %. The amount of administration of the drug was 7 injections. 60 % of the animals were subsequently transferred to another treatment regimen. In the second group, the therapeutic effect was 70 % and the number of injections was 5.

The analysis of the course of the disease showed that after 2–3 administration of the drug, contractile function of the uterus increased. At the same time, we noted that exudation increased and mucus changed its consistency. She became transparent and more viscous. However, only after 4–5 administration of the drug, the uterus contracted to a non-pregnant state.

3 Conclusion

Therefore, further studies will focus on reducing uterine rigidity which in our opinion will reduce the number of days of treatment of animals and increase the therapeutic effect.

Thus, when studying the influence of the developed drug, it was found that it does not have a negative effect on the body of laboratory animals.

A clinical study of the experimental animals' organism state after instillation of the drug did not reveal changes in the main indicators of the animals' clinical status from reference values.

The visual assessment results of the drug effect on the experimental rabbits' eyes mucous membrane indicate that, during the experiment, the drug does not cause a reaction from the conjunctiva, cornea and eyelids.

Conducted preclinical studies of the drug indicate the possibility of further clinical trials in cows with acute postpartum endometritis.

Preliminary clinical trials confirmed the effectiveness of the new preparation in a dose of 100 ml intrauterine, within 5 days.

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