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Original article

Experimental research on the use of innovative phytoextracts in cow with clinical endometritis

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Abstract

Clinical endometritis is a frequent and important pathology on farms in Romania and not only, so we created and obtained a preparation exclusively from plants in different forms of administration (tablets and intrauterine solution), with which to treat the cows under study. We chose a group of cattle that we divided into 4 batches of cows, a control batch treated with classical medication, a batch treated with intrauterine solution, a batch treated only with tablets, and the fourth batch was treated with tablets and intrauterine solution.

Based on a well-established working protocol and paraclinical investigations performed, it was demonstrated the usefulness of phytoextracts in the prevention and treatment of clinical endometritis, but also the way of stimulating the immune response associated with a shorter healing time.

Keywords endometritis; post-partum; phytoextract, cows; experimental

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Introduction

Endometritis is known as inflammatory pathology in the endometrium and results in the accumulation of purulent contents or polymorphonuclear cells (PMN). In clinical cases, in cows, it is classified into clinical endometritis or subclinical endometritis, often having a bacterial or infectious substrate. Most often they identify themselves as a result of abortions, the presence of metritis and calving dystocia, as well as in the case of fetal retention. (Fabio Lima, 2022). Opportunistic pathogens at the vaginal level or external environment should be remembered as predisposition factors, although naturally cows with an appropriate immune status can eliminate infections of a temporary nature. Inadequate or poor immunological state may be the basis of endometritis of a chronic and hardly treatable character. In cows, clinical endometritis is manifested by purulent or mucopurulent vaginal discharge 21 days after calving, and the subclinical one is characterized by a lack of secretions at the vaginal level. (Fabio Lima, 2022).

It is known that in cattle farms the management of dairy and reproductive production occupies a major interest in research, that is why we have chosen to study and get involved in the treatment of clinical endometritis. Diagnosis of post-partum diseases at the uterine level is a very important aspect for practicing veterinarians. We conducted this study based on the controversies created by its treatment and effectiveness in clinical endometritis, which has as a principle the stopping and reversal of inflammatory changes that affect fertility, as well as the increase of the immune response (S. J. LeBlanc, 2002).

Known major economic losses due to endometritis include decreased fertility, decrease in the number of products of conception, decrease in the number of parturitions, decrease in milk production, as well as premature depreciation of cattle, thus increasing production costs and decreasing profit (Haben Fesseha, 2020). Clinical endometritis is characterized by the presence of purulent-looking discharge from the uterine level and is complicated with infertility or subfertility. Their impact from an economic point of view is major, some studies recognize the decrease in the number of conception products by up to 20% compared to healthy cows and the increase in the interval from calving to conception by 30 days (Timothy J. Potter, 2010). Infertility is a biological phenomenon that is manifested by the degradation of the reproductive function in females, caused by abnormalities or diseases of the genital system or of other organs and systems, or by abnormal conditions of exploitation (improper nutrition, maintenance, environmental and climate factors) and the non-observance of the biotechnological conditions of reproduction. (M. Umer, 2022). Very often, endometritis is associated with diseases of the genital sphere (retention of fetal coverings, ketosis or specific/nonspecific uterine infections). Even during the normal puerperium in dairy cattle, multi-pathogen bacterial infection of the uterus occur, altering the immunological homeostasis and resumption of ovarian cyclicity (Kiracofe GH, 1980).

Multifactorial causes and post-partum physiology of the cervix to dilate, while the vestibule and vagina relax, make bacterial contamination inevitable and require mobilization of components with an immune role. The presence of leukocyte infiltrate and congestion of the endothelial endothelium, with or without edema, has been shown to involve the start of drug treatment. Cattle that have had such post-partum reactions, are those in which the interval between calving increases by up to 12 days or shows abortion or dystocia. (Haben Fesseha, 2020). In general, acute forms of the disease, untreated or improperly treated, take on subacute or chronic clinical aspects. Always the acute forms of the disease have an obvious character that is very easy to recognize, the subclinical or chronic forms cause subfertility or infertility (Wagener K et al., 2017).

It has been reported that the immune capability of the uterus is influenced by steroid hormones, especially luteal progesterone, which increases the susceptibility of the postpartum endometrium to infection in dairy cattle (Sheldon IM et al., 2009). Bovine endometrium cells presents Toll-like receptors which recognize and respond to bacterial infection (certain components of bacteria like LPS) leading to secretion of cvtokines, chemokines and antimicrobial peptides (Davies D et al., 2006, Menzies M et al., 2006). Subclinical endometritis defines endometrial inflammation in the absence of clinical signs (without leakage) or by the presence of over 18% polymorphonuclear (PMN) at the endometrial level between days 22 and 33 post-partum or over 10% polymorphonuclear between days 34 and 47 post-partum. The impact of subclinical endometritis on reproductive performance results from the decrease in the rate of conception and the prolongation of the service-period. Moreover, it has been shown that these types of infections negatively influence the viability and quality of embryos.

In subclinical forms of the disease, microorganisms that determine the inflammatory reaction at the level of the endometrium are most often eliminated by complex humoral mechanisms, but an active imbalance of anti- and pro-inflammatory factors at the uterine level is maintained, which will prevent the resumption of normal reproductive activity and the onset of gestation. (Iain Martin, 2017).

Materials and Methods

Materials

Based on the studies carried out by colleagues from other countries, who used as an adjuvant treatment in endometritis produced from plants (cinnamon oil, oregano,

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thyme), we created products containing fennel oil, cumin, sage, fennel, Lychnis, Achillea millefolium. The still unknown antibacterial activity of these plant products makes the study a research one. The clinical study was conducted on Holstein dairy cows, at 40-45 days post-partum, with infected uterine secretions, detected by clinical and gynecological examination in order to identify the post-partum status and conduct experimental studies in the therapy of these states with our products. Clinical examination revealed the general condition of the animal in the first postpartum month, by assessing the score of the body condition and digestive, respiratory and urinary function. The gynecological examination was performed through specific tests and investigations aimed at highlighting the specific changes in the genital apparatus, the extension of the lesions and the susceptibility of the uterus to infections with specific or nonspecific pathogenic germs.

In particular, there were appreciated the quality and quantity of the vaginal discharge as well as their appearance. Ultrasound examinations were carried out to highlight the structures on the surface of the ovaries and the appearance of the uterus. there was appreciated the amount of uterine contents, the appearance and thickness of the walls of the uterine horns and cervical canal. Being a septic inflammatory process, the content of the uterus in bacteria fluctuates greatly as a result of contamination, elimination and recontamination, due to imposed defense mechanisms. Pathogenic germs persist at the uterine level and will delay resumption of uterine function and normality. Uterine cytological examination is recommended in this situation to identify the types of pathogenic germs and test their sensitivity.

Method

In our experimental studies, there were used 3 batches, combining the 2 products, experimentally tested separately in cows with clinical endometritis. There were created 3 batches composed of 2 cows with subclinical endometritis.

We collected biological samples in cows before the experiment, 3 days, 7 days, and 14 days after the start of the experiment. The treatment was done for 7 days by combining the two forms, with the mention that the medicinal forms of the tablet type (10 tablets / animals) were administered in the form of a food bowl. Dairy animals are subject to clinical observation.

The experimental model was:

On day 1 clinical and gynecological examination in all animals taken under study. After ultrasound and clinical/gynecological examination, cows without clinical signs of endometritis (clinical endometritis) were selected in the study. On postpartum day 2, biological products (blood, secretions) are collected and medication is administered. Three different batches of cows were made in which the treatment was divided as follows:

- the first batch was considered a witness and received classical treatment (antibiotherapy and support), which was not our object of research was not included in the monitoring and results tables.
- 2. the second batch was treated only with intrauterine solution - charge 1.
- 3. the third batch was treated only with phytoextracts tablets (10 tablets / animal in the form of a food bowl) charge 2.
- the fourth batch was treated with both tablets and intrauterine solution – charge 3.

On day 3, biological samples were collected from the 3 batches of cows. On day 7, biological samples were collected from the 3 batches of cows.On day 14, biological samples were collected from the 3 batches of cows.

Discussion

According to the experimental protocol, for the combined batches of product (in the form of tablets and liquid) 2 cows with clinical endometritis were chosen. Vaginal discharge was highlighted only after transrectal examination (see picture) and have a yellowish white appearance with a strong odor (Fig 1).



Figure 1. Vaginal discharge in clinical endometritis.

Experimental results of the use of our combined products from batch 1, 2 and 3



Figure 2. Experimental products.

				Table 1.				
Paramet.	U/m	Physiology.	Cha	rge 1	Cha	rge 2	Cha	rge 3
		limits	1.1	1.2	2.1	2.2	3.1	3.2
WBC	10-9/mm ³	4-12	8,81	10,52	8,75	13,22	8,82	11,4
lym	10-9/mm ³	2,5-7,5	3,28	6,56	3,76	5,61	3,30	4,26
MON	10-9/mm ³	0-1	0,84	0,94	0	0,12	0,13	0,15
NEU	10-9/mm ³	0,6-7,6	4,62	2,65	4,75	7,18	5,19	7,3
EOS	10-9/mm ³	0,1-1	0,07	0,35	0,16	0,30	0,20	0,47
BASS	10-9/mm ³	0-0,5	0	0,01	0,01	0,01	0,01	0,01
lym	%	45-75	37,2	72,3	42,9	42,4	37,4	54,2
MON	%	2-7	9,6	9,0	0,9	0,9	1,5	0,8
NEU	%	15-45	52,4	25,2	54,3	54,3	58,8	53,8
EOS	%	1-8	0,8	3,4	1,9	2,3	2,2	2,2
BASS	%	0-3	0	0,1	0,1	0,1	0,1	0,1
RBC	10-12/mm ³	5-10	5,98	RBC	7,34	8,18	7,29	8,02
hgb	g/dl	8-15	9,3	hgb	11,3	11,9	11,5	11,8
Het	%	24-46	28,49	28,76	33,51	37,55	34,59	34,65
CVM	fl	40-60	48	52	46	46	48	45
MCH	pg	11-17	15,6	17,3	15,3	14,5	15,7	13,8
MCHC	g/dl	30-36	32,7	33,5	33,6	31,7	33,1	32,4

Table 1

The results of the hematological examination of blood samples taken on day 3 of the experiment are shown in Table 1. From the table it is noted that the hematological values are inscribed in the physiological parameters. Only in the leukocyte formula is observed in case 1.1. neutrophils with monocytosis. On day 7 of the experiment, the values shown in Table 2 were registered, showing slight changes in hematological parameters. Thus, in case 2.2 where batch 2 preparations were administered, a hyperleukocytosis with monocytosis. The data recorded at 14 days (Table 3) shows again, that the hematological examination is a little conclusive. It turns out that the 2 cows responded positively to the preparations of the batch 1. The results of the blood biochemical examination reveal important changes, recorded at days (Table 4). Changes are observed in the 2 cows throughout the experiment in terms of increased LDH, total bilirubin, alkaline phosphatase. 7 days after the beginning of the experiment (Table 5), the increase in total proteins with hyperglobulinemia is observed, the other biochemical parameters having values similar to those of day 3 of sampling. This also entitles us to perform an electrophoretic examination to elucidate which of the globulins undergoes changes and can explain, in addition to the clinical examination, the therapeutic evolution. And at 14 from the beginning of the experiment it turns out that they are the same changes in biochemical parameters (Table 6). And this time a hyperproteinemia occurs with an increase in globulin fractions.

We conducted the research between 01.05.202 and 19.05.2022.

Paramet.	U/m	Physiology.	Charge 1		Cha	rge 2	Charge 3	
		limits	1.1	1.2	2.1	2.2	3.1	3.2
WBC	10-9/mm ³	4-12	8,11	8,23	10,52	13,03	8,90	9,59
lym	10-9/mm ³	2,5-7,5	4,63	4,47	6,56	5,88	5,89	5,62
MON	10-9/mm ³	0-1	0,04	0,08	0,94	1,29	0,05	0,06
NEU	10-9/mm ³	0,6-7,6	3,21	3,10	2,65	5,02	2,63	3,42
EOS	10-9/mm ³	0,1-1	0,22	0,57	0,35	0,75	0,33	0,49
BASS	10-9/mm ³	0-0,5	0,01	0,01	0,01	0,09	0,01	0
lym	%	45-75	57,0	54,3	62,3	45,1	66,1	58,6
MON	%	2-7	0,5	0,9	9,0	9,9	0,5	0,6
NEU	%	15-45	39,6	37,7	25,2	38,5	29,5	35,7
EOS	%	1-8	2,7	6,9	3,4	5,8	3,7	5,1
BASS	%	0-3	0,1	0,1	0,1	0,7	0,1	0
RBC	10-12/mm ³	5-10	5,75	5,94	5,57	5,92	6,07	6,35
hgb	g/dl	8-15	10,4	9,7	9,6	10,0	9,1	10,2
Het	%	24-46	32,59	30,77	28,76	31,19	30,30	33,82
CVM	fl	40-60	57	52	52	53	50	53
MCH	pg	11-17	18,1	16,3	17,3	16,9	14,9	16,0
MCHC	g/dl	30-36	32,0	31,5	33,5	32,0	29,9	30,1

Table 2 :

A few days before the start of the experiment, I conducted a dosage of protein fractions in 5 control cows.

Serum protein electrophoresis is a technique that consists in the separate migration of protein fractions of serum on a semi-solid gel in the presence of an electric current. The only individual protein that was distinctly separated from electrophoresis is albumin that showed a clinical correlation only when low values from normal appeared.

The 5 samples after staining to reveal the migration of protein fractions.

Table	3 :	
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Paramet.	U/m	Physiology.	Charge 1		Cha	rge 2	Charge 3	
		limits	1.1	1.2	2.1	2.2	3.1	3.2
WBC	10-9/mm ³	4-12	8,21	10,52	8,68	8,51	8,81	6,45
lym	10-9/mm ³	2,5-7,5	3,20	5,64	4,87	4,00	3,26	3,66
MON	¹⁰⁻⁹ /mm ³	0-1	0,08	0,09	0,08	0,05	0,84	0,06
NEU	10-9/mm ³	0,6-7,6	4,40	4,37	3,31	4,33	4,62	2,58
EOS	¹⁰⁻⁹ /mm ³	0,1-1	0,53	0,41	0,40	0,13	0.07	0,14
BASS	10-9/mm ³	0-0,5	0,02	0,02	0,02	0	0	0
lym	%	45-75	38,9	53,6	56,2	47,0	37,2	56,8
MON	%	2-7	1,0	0,8	0,9	0,6	9,6	0,9
NEU	%	15-45	53,6	41,5	38,1	50,9	52,4	40,1
EOS	%	1-8	6,3	3,9	4,6	1,5	0,8	2,2
BASS	%	0-3	0,2	0,2	0,2	0	0	0,1
RBC	10-12/mm ³	5-10	5,55	5,80	5,47	6,14	5,96	5,68
hgb	g/dl	8-15	9,0	9,5	8,9	9,2	9,3	9,7
Hct	%	24-46	28,04	29,76	28,01	29,15	28,49	31,11
CVM	fl	40-60	51	55	52	47	48	53
MCH	pg	11-17	16,2	16,4	16,2	15,0	15,6	16,5
MCHC	g/dl	30-36	32	32	31,7	31,6	32,7	31,0

Table 4 :

Paramet.	U/m	Physiology.	Charge 1		Charge 2		Charge 3	
		limits	1.1.	1.2.	2.1	2.2	3.1	3.2
Protein all	g/dl	5,8-8,5	11,0	11,8	7,1	6,8	6,3	7,6
Albumin	g/dl	2,5-3,7	3,8	3,8	2,7	3,3	3,5	3,3
Globulin	g/dl	3,3-4,8	7,2	8,2	4,4	3,5	2,8	4,3
GOOD	mg/dl	10-25	13	12	9	11	13	10
UA	mg/dl	1,0-2,1	2,0	2,0	1,0	1,1	0,9	2,3
The Create	mg/dl	0,4-1,0	0,4	0,6	1,0	1,2	1,1	0,5
T-Cho	mg/dl	70-280	152	127	75	85	71	230
GOT	UI/m	78-132	42	27	68	91	92	46
ldh	UI/m	692	4000	4000	1422	1654	1637	3700
T-Bil	mg/dl	0-0,3	0,7	0,6	0,4	0,7	0,5	0,4
GPT	UI/m	0-82	17	12	9	12	7	17
ALP	UI/m	0-80	118	113	110	135	69	89

Table 5:

Paramet.	U/m	Physiology.	Charge 1		Charge 2		Charge 3	
		limits	1.1.	1.2.	2.1	2.2	3.1	3.2
Protein all	g/dl	5,8-8,5	11,0	11,2	12,4	10,5	10,4	9,6
Albumin	g/dl	2,5-3,7	3,8	3,8	3,8	3,5	3,4	3,6
Globulin	g/dl	3,3-4,8	7,2	7,4	9,6	7,0	7,0	6,0
GOOD	mg/dl	10-25	12	13	12	13	10	11
UA	mg/dl	1,0-2,1	2,0	2,0	2,4	2,2	2,0	2,1
The Create	mg/dl	0,4-1,0	0,5	0,6	0,6	0,4	0,5	0,7
T-Cho	mg/dl	70-280	154	178	244	212	123	122
GOT	UI/m	78-132	41	63	27	42	41	39
ldh	UI/m	692	4.000	3789	3560	3980	3800	3980
T-Bil	mg/dl	0-0,3	0,4	0,4	0,3	0,3	0,3	0,4
GPT	UI/m	0-82	23	20	12	17	21	18
ALP	UI/m	0-80	99	132	122	121	132	125

				10010 01				
Paramet.	U/m	Physiology.	Cha	Charge 1		rge 2	Cha	rge 3
		limits	1.1.	1.2.	2.1	2.2	3.1	3.2
Protein all	g/dl	5,8-8,5	11,0	9,6	12,2	11,3	10,4	9,8
Albumin	g/dl	2,5-3,7	3,7	3,6	4,0	3,8	3,7	3,5
Globulin	g/dl	3,3-4,8	7,3	6,0	8,2	7,5	6,7	6,3
GOOD	mg/dl	10-25	15	13	13	13	11	12
UA	mg/dl	1,0-2,1	2,8	2,0	2,2	2,3	2,1	2,1
The Create	mg/dl	0,4-1,0	0,6	0,6	0,6	0,5	0,5	0,6
T-Cho	mg/dl	70-280	160	162	142	123	133	154
GOT	UI/m	78-132	44	41	31	50	46	58
ldh	UI/m	692	3800	3650	3880	3900	4000	3800
T-Bil	mg/dl	0-0,3	0,4	0,2	0,3	0,4	0,3	0,3
GPT	UI/m	0-82	8	15	12	13	36	10
ALP	UI/m	0-80	134	122	133	138	143	149

Table 7.

Table 6:

Parameter	U/m	Physiology.	Case 1	Case 2	Case 3	Case 4	Case 5
		limits					
Protein all	g/dl	5,8-8,5	8,60	9,40	7,10	6,80	6,30
Albumin	g/dl	2,5-3,7	3,80	3,80	2,70	3,30	3,50
Globulin	g/dl	3,3-4,8	4,80	5,60	4,40	3,50	2,80

			Table 8:	:			
Fraction	U/m	Physiology.	Case 1	Case 2	Case 3	Case 4	Case 5
		limits					
Total Protein	g/dl	5,8-8,5	6,40	7,10	6,70	6,90	6,30
Albumin	g/dl	1,3-2,47	1,83	2,08	2,20	2,62	2,76
α1	g/dl	0,19-0,78	0,42	0,35	0,10	0,41	0,30
α2	g/dl	0,19-0,78	0,60	0,79	0,78	0,49	0,40
β1	g/dl	0,32-0,84	1,00	0,75	0,97	0,69	0,64
β2	gd/m	0,32-0,84	0,44	0,57	0,76	0,52	0.35
γ	g/dl	1,75-2,72	2,21	2,57	2,09	2,17	1,86
White/Globe	/	0,45-1,31	1,83:	2,08:	0,48	0,61	0,78
Ratio			4,57	5,02			

The value of protein parameters, by biochemical examinations, using kits (Table 7).

Determination of protein fractions by electrophoresis revealed the following situation (Table 8).

The most important observations on the therapeutic efficacy of the tested preparation were found in the clinical and gynecological examinations, for which we present the appearance of the vaginal discharge certifying endometrial in-



fection: on the first day of the experiment, after performing the transrectal examination, a purulent discharge appears; 10 days after the beginning of the experiment the secretion disappeared, and at the control on the 14th day, samples were collected for cytological and bacteriological examination.

Conclusions

- Depending on the severity of endometritis may be used different pharmaceutical forms created for preventive, curative purposes or with the aim of supporting classical treatment, with antibiotic.
- 2. For a better management of clinical endometritis we recommend the association of the created phytoextracts with the treatment used at the classical case.
- Following the analyzes and researches carried out, it was identified the stimulation of the cellular immune response in the studied cows, a faster healing time being

Case 1	
FACULT	Y OF VETERINARY MEDICINE BUCHAREST
28.04.202	22

vezuitate ana	aliza:			
		Total Proteine	= 6,40 g/dl F	Raport A/G = 2,19
Parametru	Rezultate (%)	Rezultate (g/dl)	Referinte (%)	$\uparrow \Lambda$
H] Alb	68,63	4,39	2738	
Alfa1	5,30	0,34	48	
Alfa2	5,64	0,36	48	
H] Beta	18,57	1,19	510	
L1 Gama	1.86	0,12	12.22	

Case 2 FACULTY OF VETERINARY MEDICINE BUCHAREST

28.04.2022

Pacient:

Varsta: Sex: ID: Rezultate analiza:

Total Proteine = 7,10 g/dl Raport A/G = 0,64

Parametru	Rezultate (%)	Rezultate (g/dl)	Referinte (%)	$\uparrow \land \land$
[H] Alb	38,96	2,77	2738	
Alfa1	5,79	0,41	48	````,
[H] Alfa2	8,48	0,60	48	
[H] Beta	13,88	0,99	510	
[H] Gama	32,88	2,33	1222	

Case 3

FACULTY OF VETERINARY MEDICINE BUCHAREST 28.04.2022

Pacient: Varsta: Sex: ID:

Rezultate analiza:

Total Proteine = 6,70 g/dl Raport A/G = 1,04

Parametru	Rezultate (%)	Rezultate (g/dl)	Referinte (%)	1. Λ
[H] Alb	51,01	3,42	2738	
Alfa1	5,54	0,37	48	1 ho d h
[H] Alfa2	8,18	0,55	48	
[H] Beta	18,20	1,22	510	
Gama	17,06	1,14	12.22	

Case 4

FACULTY OF VETERINARY MEDICINE BUCHAREST 28.04.2022

Pacient:

Rezultate analiza:

Varsta: Sex: ID:

Total Proteine = 6,90 g/dl Raport A/G = 1,24

Parametru	Rezultate (%)	Rezultate (g/dl)	Referinte (%)
[H] Alb	55,34	3,82	2738
Alfa1	5,44	0,38	48
[H] Alfa2	8,32	0,57	48
[H] Beta	14,43	1,00	510
Gama	16,47	1,14	12.22

FACULTY OF VETERINARY MEDICINE BUCHARE 28.04.2022 Pacient: Varsta: Sex: ID: Rezultate analiza: Total Proteine = 6,30 g/dl Raport A/G = 2,19 Parametru Rezultate (s/s) Rezultate (g/dl) Referinte (%) [H] Abb 68,60 4,32 2738 Afat 4,98 0,31 48 H) Beta 14,67 0,92 510 I/L 0,92 510 I/L 0,92 510 I/L 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27 0,92 1,27					3	Case
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28.04.2022 Pacient: Varia: Sex: ID: Total Proteine = 6.30 g/dl Raport A/G = 2,19 Parametru Rezultate (%) Rezultate (g/dl) Referinte (%) Plf Aba 68.60 4.32 2738 1 Atlant 4.98 0.31 48 1 PH Bba 14.67 0.92 510 1		ente beenniteb		, ETER		
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[H] Beta 14,67 0,92 510			48	0,54	8,58	[H] Alfa2
11 Camp 2 16 0 20 12 22			510	0,92	14,67	[H] Beta
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Figure 4. Vaginal discharge in first day.



Figure 5. Secretion disappeared in 10th day.

established in the animals of group 3 in which the two products (tablets and intrauterine solution) were associated in the treatment.

4. It has been found that the product has a local antiseptic effect.

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