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THE EFFECT OF HERBS EXTRACT WITH PROPOLIS AND ST JOHN’S WORTH (HYPERICUM PERFORATUM L.) ON MORPHOLOGICAL CHARACTERISTICS OF COW’S ENDOemetrium


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In the study effects of the herbs extract on the morphological characteristics of cow’s endometrium have been observed. The herbs extract have been intrauterine applied to the cows with infertility disease. The cows have been divided in to three groups. The first group of cows have been treated with intrauterine application of the extract three days before sacrifice, the second group have been treated two days before sacrifice and the first group have been treated one day before sacrifice. After sacrifice the uterine samples have been taken and treated by the standard histological procedure. The samples have been embedded in to paraffin, cut in 5 mm thick sections and stained with hematoxilline -eozin. The following morphological parameters have been studied: hiperemia, amount of exudates and width of superficial layer. The results have shown that the amount of exudates have been the highest in the group of cows sacrificed 24 h after treatment. Also in this group the highest rate of hiperemia has been noticed comparing to the other two groups. In contrast to this superficial layer width have been the largest in the group of cows treated three days before sacrifice.

The shown results indicate that intrauterine application of the herbs extract with propolis and Klamath influence the morphological changes in an endometrium, and a level of this changes depend on length of treatment.

Key word: endometrium, histology, herbs extract

Introduction
Bovine endometritis may occur after coitus, artificial insemination, normal and abnormal parturition and puerperal complications (Farca et al., 1997). Postpartum nonspecific endometritis is a common cause of reduced reproductive and production efficiency of dairy cows. In some herds, 40% of the postpartum cows may be diagnosed with, and treated for, endometritis (Steffan et al., 1984; Smith et al., 1998). Decreased productivity, increased treatment and labour costs, and increased calving to conception intervals have serious economic impacts on dairy operations (Bretzlaff, 1987; Lewis, 1997; Sheldon andNoakes, 1998).

The contemporary veterinary practice requires application of ecological medicines based on medical plants. In this way negative effects of antibiotics (resistance) and economic losses, caused by waiting period in milk usage for certain period of time after application, would be avoided. Wide number of herbs are proved to have extracts with antimicrobial and antiinflammatory effect (Lipnickij et al., 1987). In the study effects of the herbs extract on the morphological characteristics of cow’s endometrium were observed.

Material and methods
The experiment was done at the farm of 300 dairy cows (black -white Holstein-Friesian breed), kept in tied way of breeding. The farm production is about 5500 kg of milk with 3.40% milk fat. The nutrition was a complete mixed ration, composed of 4 kg concentrate, 15 kg (whole) maize silage and 6 kg lucerne hay, per cow daily. The chosen cows, suffering from chronic endometritis and prolonged service period, were treated with an emulsion of medicne plant extracts with propolis. The animals were sacrificed on the fourth day from beginning of the treatment. The emulsion had following ingredients: extracts of medicine plants (StJohn worth, margold and yarrow) 120 mg, beeswax 50 mg, pollen 10.0 mg, propolis 5.0 mg, conifer resin 5.0 mg, sunflower oil till 1.0 ml. The emulsion, warmed to 45°C, was intrauterineously applied, in dose of 100 ml with use of a catheter. The bacteriological and cytological control of uterine milk was performed before the emulsion application. From the

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experimental group, two cows were treated on the first day, two cows on the second day and two cows were treated on the third day of the experiment.

On the fourth experimental day, cows were sacrificed in a slaughterhouse. After slaughter, uteruses were taken. Uterine tissue samples were taken from the uteruses between their body and horns. The samples were fixed in Bouen's solution for 48 h, after dehydration and enlightening in xilol, samples were embedded in to paraffin. The embedded samples were cut with a microtom. Produced cuts, 5 mm thick, were stain with hematoxilin-eozine.

Histological analysis was done with use of the light microscope "LEICA DMLS" and the digital camera "LEICA DC 300".

Results and discussion

Endometrium of the cow in control group were shown at figure 1.

![Figure 1. Endometrium of the cow in control group; EP – epithelium, SL – superficial layer, DL – deep layer, GL – uterine gland, BV – blood vessel (H&E).](image1)

Endometrium of cows in control group consists pseudostratified columnar epithelium at surface. In the propria-submucosa is clear difference between superficial layer and deep layer. Uterine glands are well developed. Blood vessels are also noticed without signs of hyperemia.

Figure 2. Present endometrium of the cow sacrificed 24 h after application of the emulsion

![Figure 2. Endometrium of the cow sacrificed 24 h after emulsion application; EX – exudate, SL – superficial layer, DL – deep layer, BV – blood vessel (H&E).](image2)

The endometrium of this group of cows characterizes the presence of wider superficial layer and greater number of cells in the deep layer, in contrast to the control group. Great amount of exudate, between superficial and deep layer, is noticed as well. The blood vessels dilatation in the stroma is strongly expressed (figure 3).

In the group of animals treated with the emulsion 48 h before sacrifice, considerable blood vessels dilatation is noticed, in contrast to the control group. Greater number of cells in the both stroma's layers and only small amount of exudate are noticed as well.

The widest superficial layer is found in the group of animals sacrificed 72 h after the emulsion application (figure 5). Exudate in the stroma's superficial layers is not noticed, neither dilatation of blood vessels is noticed.

The changes, noticed 24 h after the application, indicate that the emulsion causes hyperaemia of blood vessels. Rise in amount of exudate indicates increased level of endotel capillaries passage.
Likewise the rise of neutrophils in blood vessels indicates the presence of haemoattractants. Marked blood vessels dilatation and rise in number of cells in both stroma's layers, in animals sacrificed 48 h after the application, indicate continuation of neutrophil's migration process. The absence of hyperaemia and exudate in the endometrium of cows sacrificed 72 h after the application, indicates calming down of acute inflammation. In this group of animals the widest superficial layer is observed. In this layer, rise of cell's number especially neutrophils, is noticed, comparing to the control group. Formed hi this way the layer represents a respond on intrauterineously application of the emulsion.

Above mentioned signs indicate an acute inflammatory process, which is characterized with domination of neutrophils in cell's infiltrat of an endometrium. Migration of neutrophils from blood towards superficial layer, as a defence mechanism of body against inflammation, is described in many studies (Dhaliwal et al., 2001; Zerbe et al., 2003). The mechanism of resulted changes itself, under influence of the emulsion, is not understood in detail. One of the possible answers should be allergic reaction caused by the pollen. It is known that pollen's allergens cause degranulation of mastocytes and basophils, which result in release of bioactive mediators responsible for blood vessels dilatation. Besides this, it is known that a conifer resin causes allergic reaction as well. Essential oils from the conifer resin after the application cause occurrence of bioactive materials (histamine, serotonin, etc.) (Lipnickij et al., 1987). The same authors report antimicrobial and balmy effects of propolis extracts, medicinal plants (St. John worth, marigold, yarrow) and conifer resin, which were observed by Hipokrat in V century B.C.

However, besides allergic reaction, signs connected with inflammatory process indicate an intensive action of some haemoattractant. This finding is particularly important because one of the strategies in endometritis treatment is based on intrauterineously application of certain immunomodulators. This immunomodulators, by stimulation of peripheral migration of neutrophils, act as haemoattractants (Anderson et al., 1985; Hussain and Daniel, 1992; Subandrio and Noakes, 1997).
Conclusion

Results shown in this study indicate that

1. The intrauterineously applied emulsion has mild irritative effect with signs of acute inflammation, and the mechanism itself is not fully understood.
2. On the first day after the application, blood vessel dilatation, increased exudation and leukocytes migration in superficial layer of endometrium, are observed.
3. On the second and third day the retreat of hyperaemia signs (dilatation and exudation) is noticed.
4. On the third day the widen superficial layer of endometrium is noticed, while the other signs are absent.

From everything above mentioned, arises need of further investigations aiming more precise perception of the emulsion effects on the cow's endometrium. As well as better perception of its effects on the various stages of endometritis.

Reference

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