Pre-clinical studies of reparative properties of fetoplacental peptide bio-regulators

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Abstract. Cell technology can bring veterinary medicine to a new level of development. Despite the success of modern veterinary medicine, this area is understudied having enormous opportunities and unexpected dangers. This article provides experimental data on the effect of the biologically active preparation “FPB” on laboratory animals, artificially created models of the inflammatory process. We took into account the effect of placental preparation on rat embryogenesis and embryo developmental abnormalities, since the ultimate goal of this preparation is treatment of the obstetric-gynecological pathology in dairy cows. The studies have shown that the use of drugs based on the fetoplacental complex (10%-30%) stimulates reparative and regenerative skin properties. The local irritant effect of 10% and 30% drug on the mucous eye membrane and skin of rats upon was not pronounced. In a series of experiments on reparative properties of the drug, burns caused skin hyperemia with marked exudation on the skin surface. On the sixth day of the study, the area of the burn decreased by 5.8% in the first group, by 7.7% in the second group, and by 2.4% in the control group. On the 15th day, the blisters became crusted, in 65.0% of the animals, their rejection was observed. The area of the wound decreased by 68.0%, while in the the control group, it decreased by 31.8%. The biologically active placental preparation did not have mutagenic and embryotoxic effects during the critical periods of embryo development.

1. Introduction
The development of effective drugs that suppress inflammation and adverse reactions is a relevant task. Modernization of the technological base of modern pharmaceutical production is impossible without massive introduction of biotechnologies and biotechnological products. Traditional pharmaceutical preparations, vaccines and serums contain individual biologically active substances in forms suitable for enteral or parenteral application.

A number of authors argue about the effectiveness of tissue preparations. Blood products, placenta, etc. are used [1]. Peptide preparations from the thymus gland, cerebral cortex, and the prostate gland of cattle are used [2]. It is known that regeneration processes are constantly occurring in animals. These processes are pronounced in the period of fetal development [3].

Biological substances in tissue preparations increase the resistance of the body, increase the immune response and phagocytic activity and restore the pathological process in organs and tissues. A special group of biologically active substances produced from animal tissues includes cytomedines which are peptide bioregulators that affect physiological processes (cell differentiation and proliferation, exchange and reproduction of genetic information) [4-6].
Peptide bioregulators are used for treatment and prevention. They are introduced into the body of animals as dietary supplements consisting of additional amino acids, vitamins and minerals [7,8].

Production of new drugs for prevention and treatment of inflammatory diseases of the reproductive organs is a relevant task.

Taking into account the relevance and scientific and practical significance of this problem, the purpose of our research is to study effects of the biologically active drug FPB on the organism of laboratory animals, artificially created models of the inflammatory process and their further use in obstetric gynecological pathologies in dairy cows.

2. Materials and research methods

The studies were carried out in the cell-tissue therapy laboratory of the Obstetrics and Therapy Department of Volgograd State Agrarian University. The experiments were conducted on laboratory animals (mice, rats, rabbits) in compliance with modern standards of the Ethics Committee and bioethical norms (N.N. Karkishchenko, 2010).

Raw materials for the preparation were produced from amniotic and allantoic fluids and cord blood of embryos. All raw materials were converted into the emulsified, homogeneous, liquid mass. They were settled, filtrated, homogenized, centrifugated and preserved. The raw materials were used to prepare composite preparative fractions of a biologically active drug “FPB”. A 10% fetoplacental peptide bioregulator and a 30% fetoplacental peptide bioregulator were used.

Pharmacodynamic studies were performed on laboratory animals (mice, rats, rabbits).

The effect of the FPB on the mucous eye membrane was studied on Wistar rats. In each experimental group, there were 5 rats. The conjunctival 10% drug was used for the first experimental group, and the conjunctival 30% drug was used for the second group. For the control group, distilled water was used. The drug was used once. The behavior of rats was monitored.

The irritating effect of the drug was tested on white mice. 10 drops of the 30% drug was applied on the clipped areas of skin in the interscapular region on a daily basis during twelve days. For animals of the control group, boiled liquid paraffin was applied. The animals were monitored during thirty days.

The skin resorptive action was tested on 6 guinea pigs. Animals were selected on the basis of analogs. Before the experiment, on their backs, on both sides of the spine, the hair was cut off on skin areas of 10x10 mm. The 30% drug was applied for the second group, and the 10% drug was applied for the second group. On the other side of the spine, double boiled Vaseline oil was applied.

The study of regenerative and reparative processes was carried out on rats and rabbits. In the first series of experiments, wounds were reproduced. The experiment was conducted on 15 white Wistar rats with a live weight of 180 ... 200 g. On the lateral surface of the body of each rat, the coat and skin areas of 10x10 mm2 were removed.

Animals were divided into group consisting of 5 animals. The 10% preparation was applied to the wound of the first group on a daily basis (until complete wound healing); the 30% preparation was applied to the second group, and tetracyclic ointment was applied for the control group.

The study of burn healing using the FPB was carried out as well. The experiments were conducted on 20 rats with a live weight of 200 g. Burns were made boiling water (T = 100°C), the exposure time was 10 seconds. The initial burn reading was taken with a paper template.

The mutagenic effect of the placental drug was assessed in male mice by the method of accounting for abnormal sperm heads (AGS). In the experiment, only males were used. The preparation obtained from the umbilical cord was used once at a dose of 1 ml/kg. The resulting epididymis was placed in a physiological solution and suspended. 4 drops of the 1% eosin were added to the suspension. 40 minutes after filtration through a nylon sieve, air-dry smears were prepared on a glass slide. Sperm heads were counted per 300 sperm cells.

Embryotoxic and teratogenic effects of the placental drug were assessed on adult female rats. The beginning of pregnancy was the day of sperm detection in the vaginal smear. In total, 2 series of experiments were conducted on 34 rats. In the first experiment, drug toxicity for rat embryos was assessed. For this purpose, the drug was administered intravenously at a dose of 0.05 ml/kg on the 11th
day of pregnancy. In the second experiment, the drug was administered once at a dose of 0.5 ml/kg on the 14th day of pregnancy. After the laparotomy performed on the 20th day of pregnancy, the number of corpus luteum, live and dead embryos was counted. The fetuses extracted from the uterus were studied in vivo under a binocular magnifying glass.

3. Results and discussion

When studying the effect of the "FPB" on the mucous membrane of the eye, the rats of the first group were restless; there was a slight tearing for 2-3 minutes. The conjunctival hyperemia, non-erosion and inflammation were not observed.

Animals of the second group rubbed the eyes, there were no signs of tearing or irritating effects on the conjunctiva.

Thus, the local irritant effect of the 10% and 30% drugs on the mucous eye of rats was not pronounced.

When studying the skin-resorptive effect, the erythema was observed in guinea pigs. In all animals, on the 5th and 6th days, there was an epithelium desquamation. Within 2-3 days, crusts formed on the skin surface. On the 9-10th day, young skin formed. In animals of the control group, local and resorptive manifestations were not observed during the whole experiment.

The studies showed that the 30% FPB causes insignificant epithelial desquamation and rapid irritation.

Pathological processes are often accompanied by a violation of integrity of the tissue surface. Therefore, restorative regeneration as a final stage of inflammatory diseases is of great interest. The effect of the 10% and 30% FPB in wound healing was studied as well.

The patchwork-torn wound decreased in the fifth day by 4.1 mm$^2$ in the first group, by 4.6 mm$^2$ in the second group, and by 2.0 mm$^2$ in the control group.

On the 12th day, the scab was removed in the groups treated with the FPB; the bottom of the wound was clean and pink. When treating with the 10% FPB, the area of wounds was reduced by 81.2%. Treated with the 30% FPB, it was reduced by 86.5%; in the control group, it was reduced by 65.4% (Table 1).

| Name of the group | Drug name          | Observation time, days | The period of healing, days |
|-------------------|--------------------|------------------------|----------------------------|
|                   |                    | 5  | 10  | 14  | 20  | 26  | Square, % | days |
| Control           | Tetracycline       | 26.2±4.1     | 49.2±5.1 | 62.2±3.1 | 69.9±4.2 | 93.7±5.3 | 25.9±2.2 |
|                   | ointment           |               |            |          |          |          |            |
| Experimental      | 30% PBFK           | 76.3±4.7     | 95.3±5.1 | 100.0 | - | - | 13.9±0.4 |
| group 1           |                    |               |            |          |          |          |            |
| Experimental      | 10% PBFK           | 33.7±7.2     | 78.1±4.3 | 89.1±3.1 | 100.0 | - | 20.5±1.3 |
| group 2           |                    |               |            |          |          |          |            |

In the control group, only on the 16th day, granulation tissues were formed. On the 16th day, epithelialization was observed. The wound was healed on the 24-26th day (Figure 1).

Under the action of the 30% FPB, the wound was healed on the fourteenth day; under the action of the 10% FPB, it was healed on the twentieth day. Under the action of the tetracycline ointment, the wound was healed on the 26th day (Figure 2).
When studying reparative properties of the preparations developed for treating burns, the skin hyperemia was observed in the surface layers of the skin. When touching burns, the rats were in anxious. All the rats had bubbles. On the 6th day, the area of the burn decreased by 5.8% in the first group; by 7.7% in the second group; and by 2.4% in control animals.

On the 15th day, in some rats treated with the 10% and 30% FPB preparations, the slight hyperemia was observed; opened blisters were crusted, their rejection was observed in 65.0% of animals.

When comparing the average area of wounds, it was found that in the experimental group, the area of the wound decreased by 68.0% on the 10th day. In the control group, it decreased by 31.8%.

Experimental studies did not reveal an increase in the number of abnormal sperm heads in the experimental group which indicates the absence of mutagenic effects of the FPB.
Table 2. Effects of the FPB on the embryogenesis of rats

| Group      | Number of yellow bodies | Number of embryos | Mass of one embryo, g | Number of resorbed embryos |
|------------|-------------------------|-------------------|-----------------------|---------------------------|
| Experimental | 26                      | 24                | 5.4±0.2               | 10.2                      |
| Control     | 56                      | 51                | 5.6±0.8               | 11.7                      |

The results indicate the absence of embryotoxic effects of the placental preparation. After the laparotomy performed on the 20th day of gestation, the number of yellow bodies, live and dead embryos was calculated. In the control group, the number of resorbed embryos exceeded that in the experimental groups by 1.5%.

4. Conclusion

1. The studies showed that the local irritant effect of the 10% and 30% FPB on the mucous membrane of the eye and skin of rats is not pronounced.
2. The effectiveness of wound healing using the 30% FPB was observed on the 14th day, while the effectiveness of wound healing using the 10% FPB was observed on the 20th day. In the control group, the effectiveness was observed on the 26th day.
3. The biologically active placental preparation does not have mutagenic and embryotoxic effects during embryo development.

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References

[1] Kocharyan V D, Chizhova G S, Nikitina M A 2013 A comprehensive approach to the treatment of purulent – catarrhal endometritis in highly productive cows News of the Lower Volga Agrouniversity Complex. 4(32) 136–139
[2] Kuzmin I V 2015 Prostactic peptides in the treatment of urological diseases Moscow Urologist. 1 16–17
[3] Nebogatikov G V, Frolova S P, Vershinina E A, Zakharova M A 2009 Therapeutic efficacy of stem cells and thymus preparations Questions of Regulatory and Legal Regulation in Veterinary Medicine. 4 66–67
[4] Prytkov Yu A, Varenikov M B, Chomaev A M, Artyukh V M 2008 Effectiveness of the use of a tissue preparation “Placentin-A” to increase the reproductive function of highly productive cows Scientific Achievements and AIC Equipment. 12 46–48
[5] Turchenko A N, Gorpinchenko E A, Koba I S, Strogonova T A, Vasilin V K 2008 Pharmacotoxicological assessment of tissue preparation Microbiostim Scientific J. of KubSAU. 40 196–209
[6] Fedorkin E P 2014 The role of conditionally pathogenic microflora in the occurrence of postpartum pathology of cows Sci. Bulletin Lviv Natl. Univ. of Veterinary Medicine and Biotechnology named after S Z Gzhitsky 2-1 334–339
[7] Fedotov S V, Belozertsya N S, Yakhaev I M 2016 Diagnosis and prevention of symptomatic infertility of cows. Bulletin of the Altai State Agrarian University. 11(145) 118–123
[8] Cheremnyakova L N, Buzoverov Y 2004 Hemostimulator highly effective tissue preparation Bulletin of the Altai State Agrarian University. 3 348–349