



## REVIEW

From Prof. Dinko Nedev Dinev, PhD, DVMSc, retired.

Han Tervel str. No.52, Stara Zagora,

a member of the scientific jury, appointed by order of the Rector of the University of Forestry – Sofia №369/05.07.2023 and elected as a reviewer at the first meeting of the jury.

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### On

Ph.D. Thesis with the topic: "EXAMINATION OF THE REGENERATIVE POTENTIAL OF PLATELET-RICH PLASMA AND SODIUM HYALURONATE WITH DEXPANTHENOL IN EXPERIMENTALLY INDUCED CORNEAL ULCERS IN RABBITS" for awarding the educational and scientific degree "Doctor", field of higher education 6. Agricultural sciences, professional direction 6.4. Veterinary Medicine, scientific specialty "Surgery, Radiology and Physiotherapy of animals"

**Author:** Seven Ruzhdi Mustafa, DVM, Assistant Professor at the Department of "Surgery, Radiology, Obstetrics and Gynecology" of the Faculty of Veterinary Medicine at University of Forestry – Sofia

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Seven Ruzhdi Mustafa, DVM was born on 06.12.1988 in Gabrovo. He completed his secondary education at the 18<sup>th</sup> "William Gladstone" Secondary School with intensive Chinese language study. He qualifies "professional bachelor dental technician" /2010/. During the period 2002-2007, he was a student of the FVM at University of Forestry - Sofia, who graduated with an excellent grade point average of 5.50, and the same success he showed in the state exams, with which he obtained the educational qualification degree "master". In 2019, after successfully passing the exam he was accepted for an assistant. By order of the Rector of University of Forestry - Sofia №ZSD-50 14.02.2022, he was enrolled as a Ph.D. student in a self-study form. The training was successfully completed, which is certified by the attached documentation, in particular a letter from the Head of the sector "Academic Staff" of UF - Sofia.

The thesis presented to me is oriented in an important theoretical and practical area, such as regenerative therapy. In recent years, various of its methods and application have also been studied in animals, mainly in diseases of bones, joints, and tendons. In the specific case, the author's attention is directed to the cornea, which is often subjected to various external influences and diseases, including corneal ulcers. This anatomical component of the eye enables direct visualization of changes in it, as well as the relatively easy application of drugs and reporting of the effect of the treatment.

The thesis is written in a volume of 164 pages and is structured according to the requirements for this type of work – content - 2 pages, used abbreviations – 2 pages, introduction - 2 pages, literature review - 39 pages, own research - 12 pages, results - 41 pages, discussion - 30 pages, findings - 1 page, contributions – 1 page, practice recommendations - 1 page, scientific publications related to the dissertation work - 1 page, citations - 1 page, bibliography - 26 pages. The individual sections are in a tight ratio.

The literary index includes 336 authors, of which 9 – in Cyrillic and 327 – in Latin, which allows the precise formulation of the scientific hypothesis and analysis of the obtained results.

The dissertation is illustrated with 57 figures, most of them in color, and 19 tables.

The *literature review* is purposeful and covers the most important points corresponding to the topic of the dissertation – nature of the ulcer, classification, clinical findings, features in the healing process, the main diagnostic and therapeutic approaches are indicated, in particular modern regenerative therapies. Finally, a justification is made for future intentions with the development of the dissertation work.

The remarks I made at the meeting of the primary scientific unit, mainly regarding the inclusion of some authors, have been taken into account and removed.

All results were subjected to statistical analysis by two-way analysis of variance ANOVA, and their further processing was carried out with the test of Fisher and Friedman's non-parametric test.

The main aim of the study was to make a comparative assessment of the speed and degree of healing of experimentally induced corneal ulcers after the application of autologous

platelet-rich plasma, sodium hyaluronate with dexpanthenol and a surgical method for temporary fixation of the third eyelid.

I define the scientific idea for the development of this work as modern and up-to-date.

It concerns a purely experimental study of 28 rabbits, aged 12-18 months, of the New Zealand white breed. All mandatory procedures were carried out before the start of the experiment – the same care sheet, feeding, and deworming were carried out. There is a permit for the use of experimental animals from the BFSА №337/2022.

After appropriate anesthesia, corneal ulcers were induced chemically and traumatically, forming the two main experimental groups I /chemical/ and II /traumatic/, described in detail in the dissertation work. In each main group, 4 subgroups were formed with different treatments – one control (suturing of the third eyelid) and three working groups treated differently – sodium hyaluronate and dexpanthenol as eyedrops, platelet-rich plasma as eyedrops, and the same by a single subconjunctival injection.

I find the division of the two experiments into "two stages I and II" unfortunate. A stage is a period of the same experiment, and here we are talking about conducting two separate experiments, and not continuing those already begun on the same subject. In this specific case, a chemically ulcer is induced in one eye and after a while, a traumatic ulcer is induced in the other.

Regarding the experimental design, we also come to one inaccuracy in this thesis – the so-called POSITIVE CONTROL GROUPS /MNF-Ch and MNF-Tr/, both related to suturing of the third eyelid. Control groups, if indeed necessary, should be without any treatment. In this case, the group with the fixed third eyelid is an ordinary group, like the other three. After all, this is how it is commented on – as a separate group with which all others are compared. My specific suggestion during the approbation was to remove the term "control group" everywhere in the dissertation, which the author did not comply with!

To objectively report the changes occurring after the applied treatment, a number of both classical and special methods were used, and I would like to note the biometric measurements of the anterior segment of the eye by **optical coherence tomography /AS-OST/, taken with a CASIA 2 device (Toney Corporation, Japan)** with an impressive 50,000 axial scans per second. This enables a two-dimensional image of all components of the front of the eye, with measurements of areas, angles, tissue thickness and layers. At the same time, the partial results are convincing, and the quality of the applied evidentiary material is very good. This is the first

research of this nature in the field of veterinary ophthalmology in the country, which I highly appreciate.

As a new approach to veterinary medicine, I also highly appreciate the analysis of the fluorescein dye test results using the software program **NIH /National Institutes of Health, USA/ Image version 1.54d.**

The results of the conducted research are presented in 53 figures and 15 tables.

Corrected an omission pointed out in the preliminary meeting related to the lack of explanations under the figures with the results of the 3-D reconstruction and the gonioscopic views, as well as those with the histological results /fig. 22-57/.

Based on the presented results and the discussion, 7 findings, 6 original and 4 confirmatory contributions were made.

The most important original contributions made with this Ph.D. Thesis are:

1. A complex experimental study was conducted in chemically and traumatically induced ulcers of rabbits treated with autologous platelet-rich plasma, sodium hyaluronate with dexpanthenol, and suturing of the third eyelid.

2. For the first time in native veterinary ophthalmology, optical coherence tomography was used, allowing detailed monitoring of the state of the anterior eye segment, mainly in terms of sphericity and smoothness.

3. Autologous platelet-rich plasma stimulates the healing of ulcers, regardless of the etiological factor, and in principle, ulcers of traumatic origin heal faster than those of chemical origin.

I fully accept the confirmatory contributions and recommendations for the practice,

In connection with the dissertation, 3 articles were published, all three in English. The two are from the annual congresses held in Jundola "Tradition and Modernity in Veterinary Medicine", and the third – in the online journal Advance Research Journal of Multidisciplinary Discoveries.

**The abstract**, attached to the main documentation, outlines the main achievements made with this dissertation and reflects its content.

There is no evidence of plagiarism in the dissertation.

I also have the following **remarks, recommendations, and questions** regarding the dissertation presented in this way:

1. When formulating the main goal, it is written that the speed and degree of healing will be evaluated, which is incorrect. The time and quality of healing should be evaluated.

2. For the titles of tab. 5 and tab. 6 the term "qualitative analysis" should be replaced by "quantitative analysis", since it is about counting cellular elements in the plasma.

3. When describing the experimental design, it is indicated that all animals were treated for 5 days with Meloxicam subcutaneously. It is known that NSAIDs inhibit prostaglandin production affecting platelet adhesion. Does this affect the amount of platelets in the plasma?

4. Is the instillation of PRP three drops per day equivalent to a single subconjunctival injection of 0.7 ml in terms of effect?

5. Explain the term "positive control group" that you use for the groups with fixed third eyelid.

6. The original contribution No. 5 related to the preparation of platelet-rich plasma should be cited as a confirmatory one, since this has already been done / Dissertation G. Marinov, 2017/.

### CONCLUSION

In conclusion, I believe that, despite the notes made, the dissertation completely fulfills the requirements of the Law and the Regulations for the Development of the Academic Staff of the University of Forestry – Sofia for this kind of development and has prepared the Ph.D. student for independent scientific work. At the same time, significant theoretical knowledge was acquired in the field of veterinary ophthalmology. I highly appreciate the appropriate and well-developed scientific hypothesis, experimental design, applied modern methods, the conclusions drawn, the original and confirmatory contributions.

All this gives me a reason to give my positive vote for the award of the educational and scientific degree "Doctor" of Seven Ruzhdi Mustafa, DVM, Assistant Professor in the Department "Surgery, Radiology, Obstetrics, and Gynecology" of the FVM at University of Forestry – Sofia, in the field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.4. "Veterinary medicine", and scientific specialty "Surgery, Radiology and Physiotherapy of Animals".

**03.08.2023**  
**Stara Zagora**

**Review prepared by:**

**(Prof. Dinko N. Dinev, PhD, DVMSc)**