Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 6 June 2024

Research Article

Pathophysiological Mechanisms of the Therapeutic Effectiveness of Oxidized Dextrane in Violations of Spermatogenesis of Infectious and Inflammatory Genesis

EG Novikova[1,2], AV Troitsky[1]*, TN Bystrova[1], EH Churina[3,4] and OI Urazova[3]

[1]Federal Research Centre for Fundamental and Translational Medicine, Novosibirsk, Russia

[2]Regional Medical and Diagnostic Center, Novosibirsk, Russia

[3]Federal State Budget Educational Institution of Higher Education "Siberian State Medical University" of the Ministry of Health of the Russian Federation, Russian Federations, Tomsk, Moscow Tract, Russia

[4]Federal State Autonomous Educational Institution of Higher Education "National Research Tomsk State University" 4; 634050, Tomsk, Russia

***Corresponding Author:** AV Troitsky, Federal Research Centre for Fundamental and Translational Medicine, Novosibirsk, Russia.

DOI: 10.31080/ASMS.2024.08.1834

Received: April 23, 2024

Published: May 27, 2024

Abstract

Currently, among the etiological factors of impaired spermatogenesis, infectious and inflammatory processes are considered to be the most common. And this applies not only to urogenital infections, but also to any chronic infectious processes caused by both bacteria and pathogenic fungi and viruses.

Keywords: Immunity; Fertility; Herpes

The correlation of male fertility with herpes, which initially occurs in chronic form [1], has already been established. There is evidence of impaired spermatogenesis following acute respiratory viral infections, including COVID-19 [2,3]. In the number of intracellular infections that have a negative effect on spermatogenesis should be noted, first of all, tuberculosis and candidiasis [4-6]. Despite the morphological diversity of infectious agents causing disorders of spermatogenesis, from the simplest viruses to pathogenic fungi, their general nature of influence on the immunological status of the body can be distinguished. In particular, chronic inflammatory processes occurring against the background of increased sensitization to the autoimmune component in combination with a reduction in the natural mechanisms of immunobiological resistance to infections, in particular the reduction of the activity of the cellular link of immunity. The latter is mainly due to a decrease in phagocyte activity of macrophages and incomplete phagocytosis due to the infection agent's blocking of the phagosome-lysosomal fusion in macrophages. If you consider that macrophages are able, depending on the microenvironment, to change their phenotype from M1 (induction of predominantly pro-inflammatory cytokines) to M2 phenotype (induction of primarily anti-inflammatory cytokines), it becomes obvious that the polarization of the microfugal link of cellular immunity can be a key link in violations of spermatogenesis of infectious and inflammatory genesis. In this case, the mechanisms of impaired spermatogenesis can be both systemic in nature under the influence of the cytokine profile of the blood, and the local status at the level of the herminative epithelium of the testicles. As you

know, testicular macrophages are an important link in the process of spermatogenesis, directly affecting the spermiogenesis. And in this aspect, the polarization of testicular macrophages phenotypes under the influence of the systemic character of cytokine profiles altered as a result of the infectious and inflammatory process can play a dominant role in the violation of spermatogenesis [7]. If you consider that NO is a stimulator of the production of testosterone by the Leydig cells, it is obvious that the decrease in the activity of the population of macrophages in general and testicular macrophages in particular, is the main pathophysiological mechanism of chronization of the infectious-inflammatory process and its negative effect on spermatogenesis [8]. In this regard, the search for effective and selective macrophage activators is very promising from a pathophysiological point of view. As a promising pharmacological candidate for the role of dynamic macrophage polarization activator, oxidized dextrane was of interest to us. The oxidized form of dextran exhibits high anti-inflammatory activity, but it not only enhances the phagocyte activity of macrophages, but also increases the frequency of phagosomal-lysosomal fusions in macrophages, which is extremely important for the effective fight against intracellular infections. The oxidized form of dextran is the main biologically active component of the drug "Androexpert", which according to available clinical observations is an effective means of treatment of calculous prostatitis and restoration of spermatogenesis, especially against the background of transmitted infectious and inflammatory diseases [9]. The oxidized form of dextrane showed high therapeutic and prophylactic activity in experiments *in vivo*, in the systemic inflammatory process induced by intra-abdominal administration of bacterial endotoxin (lipopolysaccharide *Esherichia coli*) [10]. There is evidence confirming the effectiveness of oxidized dextran in tuberculosis, candidiasis and viral diseases. However, there is currently no unified concept of the mechanism of the pharmacological effects of oxidized dextrane. It is assumed that the trigger mechanisms of macrophage activation by oxidized dextrane are mediated through beta-glycan receptors localized in the cytoplasmic membrane. However, this mechanism can occur at the level of resident macrophages and blood-circulating macrofages. At the seminal level, an additional mechanism of activation of testicular macrophages is possible due to the interaction of oxidized dextrane with the asyaloglycoprotein (galactose) receptors of macrofages, since the oxidised dextran, along with carbonyl groups, contains carboxylic groups of the type of sialic acids. As a model for the study of the characteristics of polarisation of macrophage phenotypes in chronic infectious and inflammatory processes, we have used immunobiological data obtained from patients with tuberculosis, including drug-resistant forms. This model allows the most comprehensive, in relation to macrophage phenotypes, to assess the main pathophysiological features of the course of chronic infectious and inflammatory process with intracellular persistence of the pathogen of the disease, which includes not only Micobacterium tuberculosis, but also Candida albicans, Ureaplasma species, Micoplasma haemofelis, as well as almost all viruses.

The objective of this paper is to summarize experimental and clinical data on the pharmacological properties of oxidized dextrane within the concept of macrophage polarization to justify the mechanism of the therapeutic action of Oxidizeddextrane in disorders of spermatogenesis.

Materials and Methods

Clinical trial on macrophage polarization in tuberculosis patients

Venous blood samples of 47 patients with tuberculosis were used as material for the study, 17 of whom had drug-resistant forms. For the isolation of monocytes from whole blood in order to transform them into macrophages, the method of centrifugation in the ficol gradient with a density of 1,077 g/cm3 was used, followed by immunomagnetic separation of CD14 + cells. The monocytes were cultivated in a full X-VIVO 10 nutritious medium with gentamicin and phenolic red with the addition of macrophage-stimulating factor (M-CSF) (5 ng/ml) at a concentration of 1×10^6 cells/ml containing stimulants such as interleukin (IL) 4 (10 ng/mL) and interferon (IFN) γ (100 ng/ml). The macrophages were immunophenotyped using monoclonal antibodies to CD80, CD86, HLA-DR, CD163, CD204 and CD206 on the Beckman Coulter CytoFLEX LX flow cytometer. The data obtained were analyzed using the software application "CytExpert 2.0". The baseline levels of both IL-4 (10 ng/ml; PeproTech, USA) and IFN- γ (100 ng/mL; PeproTech, US) stimulated secretion levels of IL-1β, IL-6, IL. -10, TGF-β were determined by ELISA immunoenzyme analysis. The results were analysed using statistical methods.

Clinical study to evaluate the therapeutic efficacy of oxidized dextran as a selective macrophage activator

The study included 52 patients with complaints of absence of conception in a couple for more than 1 year and with the latent phase of chronic prostatitis, when there were no general signs of the inflammatory process (temperature response, in blood tests: leukocytosis, acute phase indicators), but there was an increase in the number of white cells in the secretion of the prostate and ejaculate associated with patospermia. Age: 32±2.2 years.

Patients were examined according to the generally accepted scheme, including information on complaints collection, history data, general examination and physical methods: the main vital functions of the respiratory organs, circulation, gastrointestinal tract were evaluated (all of them were within the age limit), as well as – transrectal finger examination of the prostate and palpatory scrotum examination (as part of objective urological examination).

Patients were divided into two representative groups: the 1st group of patients received oxidized dextran rectally 1 time per day for 10 days, then 20 days every day; the 2nd (comparison group) received similarly a placebo in the form of rectal suppositories consisting of cocoa butter.

Two months after the start of therapy, the patients received a spermogram.

The evaluation of the parameters of the ejaculate was carried out by the international network of independent clinical and diagnostic laboratories in Novosibirsk "CITILAB" according to the recommendations of WHO 2010 [11]: were described and analyzed indicators of the spermogram, marked by the dynamics of their values during the treatment process: volume of ejaculation (ml), concentration of spermatozoa (mln/ml), total number of sperms in the ejaculate (milln), the number of leukocytes (mln/mL), the severity of agglutination sperm (cf. The study was approved on 31.05.2021 (Excerpt from the Minutes of the meeting of LEK RMDC No.2/2021 dated 31.05.2021).

Patients were included in the study with informed consent and in accordance with the ethical guidelines of the Helsinki Declaration (WMA, Edinburgh, Scotland, 2000), subject to explanatory note 29 approved by the WMA General Assembly (Washington, 2002).

Methods of statistical processing are chosen depending on the type of random values. The Shapiro-Wilk criterion was used to assess the type of distribution of characteristics. The results of the analysis of continuous values are presented in the form of M±m, where M is the selective average and m is the standard error of the average. The values of qualitative characteristics are presented in the form of observed frequencies and percentages. In the case of normal distribution, as well as equality of dispersions, the t - criterion of the student was used to compare the averages. The equality of dispersions was measured by the F-criterion of Fischer. For the comparison of the related samples, the Steward's paired t-criterion was used. The critical level of statistical significance was 0,05. The comparison of qualitative characteristics in small groups of observations was carried out using Pearson's $\chi 2$ criterion for quadrupole coherence tables. The processing and graphical presentation of the data was carried out with the help of computer programs Statistical 12.0 Corporation Stat Soft (USA) and Microsoft Office Excel, 2017 (USA).

Experimental study to evaluate the effectiveness of oxidized dextran on the LPS model of induced impaired spermatogenesis in rats

The study was carried out on sexually mature males of the Wistar line of rats weighing 180-200 g. The animals were divided into 3 groups of 5 animals: 1 group of male rats with a single intraperitoneal administration of 2 ml 0.9% NaCl, 2 group of males with a one-time intraperitoneal administration of *E. coli* at a rate of 50 μg/kg body weight, 3 group of females with a One-time intrateritourinary administration of 50 mcg/kg body weight and 2 ml of 2% oxidized Declan solution.

The animals were withdrawn from the experiment by an etheric anesthesia overdose for 3 days after the injection of the test substances, in accordance with the principles of humanity of the European Community Directives (86/609/EEC) and the Helsinki Declaration and in conformity with the requirements of the rules of conduct using experimental animals.

The object of the histological study were fragments of the testicles of rats of the Wistar line, which were fixed in a neutral 10% buffer solution of formalin with subsequent sampling, the manufacture of paraffin blocks and histological glasses with cuts of testicular tissue, dyed with hematoxylin and eosin.

The structural changes of the testicles were visualized by direct light microscopy with an enlargement of x200. Morphometric evaluation of histological structures was carried out in cross-cuts of curved seminal tubules with the most advanced stages of spermatogenesis in 10 arbitrary fields of view with an increase of x400 using a 100 point morphometry mesh of an area of 3.64x104 μm^2. This was done by calculating the numerical density (Nai) of sperm in the lumen of the tube, early and late spermatozoa, spermicides, spermatogonium, Certoli cells, Leydig cells as well as the numeric density of the vessels of the peritubular zone of the testicles, the volume-density (Vv) of the interstitiation between the curved seminal tubes and the volumetric densities of the cell infiltration.

The histological samples were examined using a digital laboratory light optical fluorescent phase polarization microscope Olympus CX43 (Japan) with image analysis and photo archiving software. The preparations were photographed with the digital camera MOTICAM S6 (Japan) with subsequent image processing.

In addition, the index of maturation of sperm cells was determined – the ratio of the sum of young (spermatogonia's, spherocytes) and mature forms (spermatozoa, spermatogenesis) of the sperm epithelium.

The statistical processing of the data received was carried out with the help of the programs Statistical and Excel. The reliability of statistical differences between indicators of similar parameters was assessed using the Stewart t criterion. Differences at the significance level $p \leq 0,05$ were considered reliable.

Results and Discussions

In part I of the clinical study, it was found that the severity of the tuberculosis process depends on the polarization of the macrophages. Thus, in the study of the cytokine profile of macrophages, a decrease in the number of cells expressing the HLA-DR activation marker was demonstrated in the transformation *in vitro* of CD14 + monocytes of blood into macrophages in patients with tuberculosis, which indicates a functional phenotypic polarization of macrophages in the direction of M2, as well as in M2 direction. This ability is independent of *in vitro* cell induction conditions (M1-INF- γ activation or M2-IL-4 activation), the clinical form of the disease or drug sensitivity. We found that *in vitro* non-stimulated and induced INF- γ (M1-activation) and IL-4 (M2 activation) secretion of IL-10 and pro-inflammatory cytokines (IL-1β, IL-6) macrophages in tuberculosis patients was higher than in healthy donors. At the same time, the secretion of TGF-β *in vitro* varied in different directions depending on the type of macrophage induction; secretion increased with M1-stimulation and decreased by M2-cell stimulation. The highest levels of non-stimulated *in vitro* secretion of IL-10 and TGF-β were observed in patients with DTB. In addition, in patients with LDT and drug-sensitive tuberculosis (LDT-TB) during macrophage transformation *in vitro*, high secretion of immunosuppressive cytokines IL-10 and TGF-β is positively correlated with increased expression of CD163, CD204, CD206 squander receptors in cells, which corresponds to the regulatory M2 macrophage phenotype. In TB patients with drug-resistant (LA) tuberculosis, *in vitro* macrophage transformation shows high levels of expression of squangler receptors and CD80/CD86 stimulation molecules on cells due to hypersecretion of cytokines with pro-inflammatory action (IL-1β, IL-6) and anti-inflammatory activity (IL-10, TGF-β) indicates a polarization of macrophage differentiation in a subpopulation of cells with M1 and M2 phenotypes. Thus, it was confirmed that the immunopathogenesis of differentiation of macrophages *in vitro* in cells with anti-inflammatory (M2) or mixed (M1/M2) phenotype in pulmonary tuberculosis is due to the secretion of pro- and anti-inflammatory cytokines, whose activity depends on the clinical form of the disease, as well as sensitivity to the drug.

Of course, first of all, this phenomenon relates to the circulating in the systemic bloodstream population of macrophages. However, it should be noted that the cytokine profile of the blood serum may directly affect the activity of resident macrophages in the organs and testicular macrophages in particular and the results obtained may explain the degree of impaired spermatogenesis depending on the severity of the disease in tuberculosis patients, taking into account the cellular activity of the immunity link.

In the second phase of the clinical study, it was found that the oxidized form of dextran, which is a selective receptor-mediated activator of macrophages, part of the drug "Andro expert", is a very effective means of restoring spermatogenesis in patients with calculated prostatitis. As is known in the genesis of calculous prostatitis, the infectious-inflammatory process is the classic trigger mechanism of inflammation of the prostate gland, which in dynamics leads to a violation of spermatogenesis and fertility.

Ejaculation volume increased in group 1 patients from 2.54 ± 0.15 to 3.13 ± 0,18 (ml), which was 23.23%; *p < 0,05 (Figure 1).

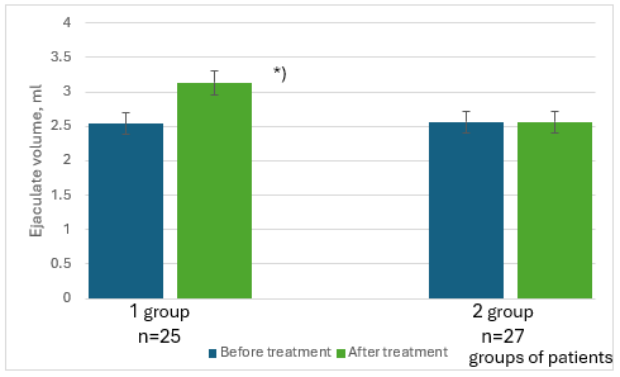

Figure 1: Dynamics of ejaculation volume indicator (ml) in evaluating the results of spermogram in groups of patients to the end of treatment.

The sperm concentration increased from 26.40 ± 4.18 to 41.12 ± 5.37 (millions/ml) - by 55.75%; p< 0.05 (Figure 2), which resulted in an increase of the total sperm count in the ejaculate by 88.5%, from 65.47 ± 8.41 to 123.41 ± 12.33 (million pieces); *p < 0.05 (Figure 3).

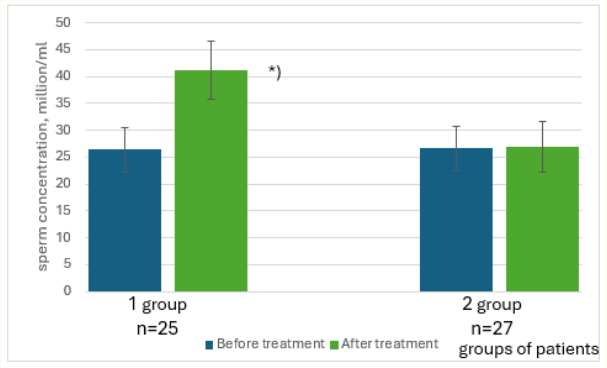

Figure 2: Dynamics of the indicator of sperm concentration (ml/ml) in the evaluation of the results of the spermogram in groups of patients to the end of treatment.

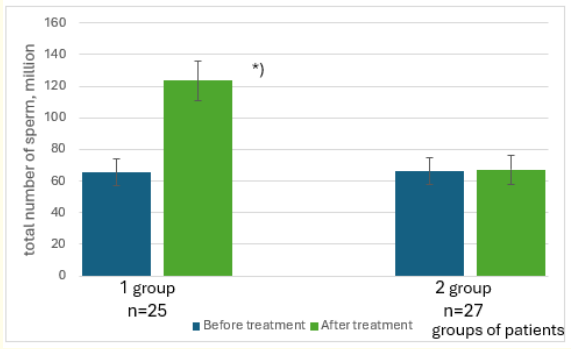

Figure 3: Dynamics of total sperm count (million) when evaluating spermogram results in groups of patients by the end of treatment.

The number of leukocytes in the same group decreased by 2.8 times: from 1.25 ± 0.17 to 0.44 ± 0.010 (millions/ml) – by 64.80%; *p < 0.05 (Figure 4).

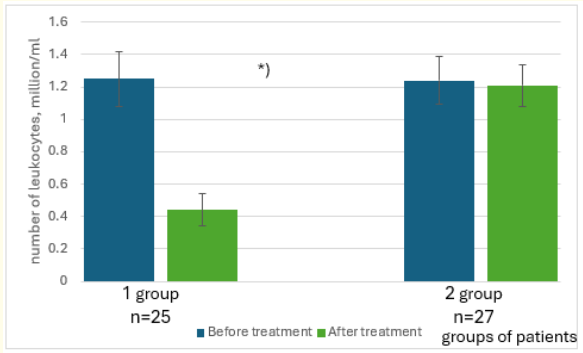

Figure 4: Dynamics of the indicator of the number of leukocytes (millions/ml) in the evaluation of the results of the spermogram in groups of patients to the end of treatment.

The severity of sperm agglutination decreased by 3 times: from 0.52 ± 0.07 to 0.17 ± 0.03 – by 0.35 units, which was 67.31%; *p < 0.05 (Figure 5).

The positive effect of "ANDROEXPERT SV1" candles on spermatogenesis in men with chronic prostatitis associated with secondary infertility is probably due not only to the anti-

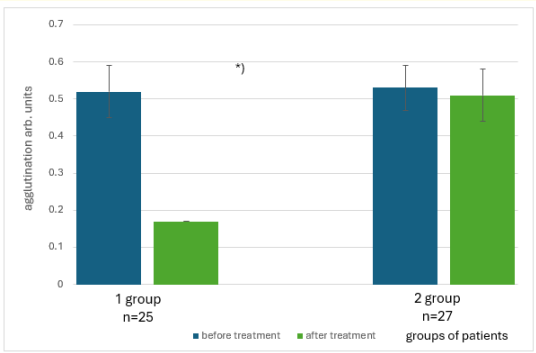

Figure 5: Dynamics of the indicator of agglutination of seminal fluid (e.g.) in the evaluation of the results of spermogram in groups of patients to the end of treatment.

inflammatory activity of oxidized dextran, but also its indirect "gonadotropic effect". This effect is not due to the stimulation of sperm proliferation, but to the improvement of the trophy of the tissues of the reproductive organs. In infectious and inflammatory processes and chronic inflammation induced by them, the normal trophy of the tissues of the prostate and testicles is disrupted and, as a result, changes in the ejaculate and disruption of spermatogenesis. Key pathogenetic factors of patospermia are the formation of microthrombus in the capillary circulation of the testicles, hypoxia and the generation by the cells of a large number of active forms of oxygen, having a detrimental effect on cell membranes. Under such a microenvironment, normal spermatogenesis is not possible. Oxidized dextrane through glycan receptors activates macrophages localized in the venous lymphatic circulation of the small pelvis, including the activation of testicular macrophages. As a result of this activation, testicular macrophages are able to more effectively phagocyte pathogenic microorganisms that support chronic inflammation, express and release anti-inflammatory cytokines, including tissue proteinases, into the extra-cellular environment, which have a thrombolysis effect and restore normal blood circulation in the capillaries, eliminating hypoxia and reducing the production of toxic active forms of oxygen [12]. It has also been found that activated testicular macrophages release 2.5-hydroxycholesterol, a steroid that can be converted into testosterone by Leydig cells [13] and thus improve their spermatogenic function.

One indicator of the viability of sperm is the degree of agglutination, which depends on the quality of seminal fluid and the immune status of the sperm itself. A substantial decrease in agglutination, along with an improvement in the above indicators, indicate an increase in the secretory activity of the sex glands, an improved process of spermatogenesis and an overall increase in male fertility.

The decrease in the number of leukocytes in the seminal fluid of patients of the same group by the end of the course indicates the presence of anti-inflammatory activity of oxidized dextrane, which was shown by us earlier in experiments on animals (in the simulation of prostate hyperplasia and bronchopulmonary pathology) [10,14] and is due, mainly, activation of macrophages.

In an experimental part of the study on a model of impaired spermatogenesis, in rats of the Wistar line, induced by *E. coli* LPS, it was found that oxidized dextran has high preventive activity, reducing the morphological criteria of impatient spermiogenesis (Table 1).

Table 1: Structural changes in the testicles of white rats after intravenous administration of *E. coli* LPS and their prevention by interventional administration of 2% oxidized dextran solution (M ± m).

Options	Study Groups		
	1 group	2 group	3 group
Number density of convoluted seminiferous tubules, Nai	30,83 ± 1,37	29,94 ± 1,86	32,98 ± 1,86
Sertoli cell number density, Nai	20,89 ± 1,26	17,52 ± 1,24$^{\alpha}$	18,87 ± 1,45
Leydig cell number density, Nai	11,23 ± 1,39	10,75 ± 1,15	13,87 ± 1,47$^{\beta}$
Spermatocyte number density, Nai	38,74 ± 1,26	29,80 ± 1,25$^{\alpha}$	37,29 ± 1,47$^{\beta}$
Number density of spermatogonia, Nai	48,97 ± 1,35	39,71 ± 1,53	49,34 ± 1,38

Spermatid numerical density, Nai	33,14 ± 1,52	24,81 ± 1,46$^{\alpha}$	27,79 ± 1,74$^{\alpha}$
Numerical density of sperm in the lumen of the tubule, Nai	300,87 ± 15,23	239,71 ± 11,35$^{\alpha}$	299,64 ± 13,32$^{\beta}$
Numerical density of vessels in the peritubular zone of the testes, Nai	7,98 ± 0,62	9,28 ± 0,74	11,21 ± 1,23$^{\alpha}$
Volume density of intertubular interstitial tissue, Vv	8,25 ± 0,87	12,74 ± 1,24$^{\alpha}$	10,47 ± 1,2$^{\beta}$
Volume density of cellular infiltration of the interstitium between convoluted seminiferous tubules, Vv	___	5,64 ± 0,32	7,12 ± 0,46$^{\beta}$

Note: α - the reliability of the differences of values of similar parameters from the indicators of group 1; β - the probability of differences in values between the parameters of groups 2 and 3. At p≤0,05.

Evaluating the integral histological picture for all 3 groups of animals and the morphometric data presented in Table 1, it can be stated that after 3 days after intarperitoneal administration of *E. coli* in rats there is a marked tendency to decrease all basic parameters of spermatogenesis in combination with elements of aseptic inflammation in the form of swelling of the interstitial, full blood and cell infiltration. These changes are moderate due to the fact that the experiment used a low dose of *E. soli* LPS, which does not lead to fatal disorders of spermatogenesis, but allows to assess the dynamics of the pathological process when it is corrected by oxidized dextran. Thus, in rats of group 3, who were given 2% oxidized dextran solution immediately after intraperitoneal administration of *E. soli* LPS, compared with animals of group 2, all indicators of spermatogenesis were higher. This trend is most evident in the sperm density indicator in the lumen of the tubulous, which in the 3rd group of animals is almost close to the value in the 1st group and significantly higher than in the animals of the 2nd group. At the same time, it should be noted that in animals of group 3, compared to animals of the group 2, the morphological manifestations of hemo circulatory disorders in the form of edema interstitial are less pronounced. However, the volume density of interstitial cell infiltration between the curved seminal tubes and the numeric density in the seminal zone of the testicles in groups 3 are, on the contrary, slightly higher than in groups 2, which may indicate an improvement in local blood flow and activation of resident testicular macrophages in response to the induced intraperitoneal administration of E. coli. Such dynamics may indicate that oxidized dextran has a moderate anti-inflammatory effect in experimental conditions, which has previously been studied in detail in various *in vivo* models. It should be noted that in this model *in vivo* the impaired spermatogenesis is not direct, but to a greater extent, indirect, due to an acute immunological response to intraperitoneal administration of *E. coli* LPS by type of systemic aseptic inflammation.

Conclusions

Improved experimental and clinical data allow to evaluate the possibility of using oxidized dextrane as a promising means of prevention and correction of disorders of spermatogenesis caused by the action of infectious agents of bacterial and/or viral etiology. As the most likely pathophysiological mechanism of the effectiveness of oxidized dextrane in disorders of spermatogenesis can be considered its ability to receptor-mediated activation of macrophages, which can be accompanied by their phenotypic polarization, determining the positive dynamics of the clinical course of chronic infectious and inflammatory processes associated with andrological pathology. However, the mechanisms of implementation of the positive protective properties of oxidized dextrane in respect of disorders of spermatogenesis due to testicular tissue damage by pathological agents of infectious etiology need further comprehensive detailed study.

Bibliography

1. Kovalyk VP., *et al*. "The role of herpesviruses in male infertility'. *I.M.G. Medical examination* 5.3 (2021): 123-129.

2. Demiashkin GA., *et al*. "Effects of SARS-CoV-2 infection on male and female reproductive systems (meta-analysis)". *Medical Journal of the Caucasus* 15.4 (2020): 582-586.

3. Adamyan LV., *et al*. "The influence of COVID-19 and other viral infections on male fertility (review of literature)". *Reproductive Problems* 26.6 (2020): 77-82.

4. Kulchavenya EV. "Tuberculosis of the prostate gland and sexual problems in tuberculosis". *Urology Journal* 3 (2014): 20-49.

5. Shcherban MN. "Effects of anti-tuberculosis and prostatotropic therapy on the reproductive system of men suffering from pulmonary tuberculosis". *Siberian Medical Journal* 2 (2008): 42-44.

6. Burrello N., *et al*. "Candida albicans experimental infection: effects on human sperm motility, mitochondrial membrane potential and apoptosis". *Reproductive BioMedicine Online* 18.4 (2009): 496-501.

7. Saposhkova JY., *et al*. "Laboratory diagnosis of male infertility. Markers and methods. Part II // Laboratory and Clinical Medicine". *The pharmacy* 1.2 (2021): 65-79.

8. Wanpeng Yu., *et al*. "Estrogen promotes Leydig cell engulfment by macrophages in male infertility". *Journal of Clinical Investigation* 124.6 (2014): 2709-2721.

9. Novikova EG., *et al*. "The use of rectal suppositories with oxidized dextrane in calculated prostatitis". *Urology* 6 (2021): 1270129.

10. Troitsky AV., *et al*. "Prospects of the use of oxidized dextran for the prevention of viral pneumonia and pulmonary fibrosis". *Medical Alliance* 9.4 (2014): 45-53.

11. WHO Laboratory Guide to the Research and Treatment of Human Sperm. 5th ed., 2010. Per. with Angl. N. P. Makarov. Scientist. ex. L. F. Kurilo. Moscow: Capital Print, 2012. (WHO Guide to Human Ejaculate Research and Treatment, ed. 5, 2010. Per. with Angl. N.P. Makarov. Scientific editor L. F. Kurilo. M.: Capital Print, (2012).

12. Figueiredo AFA., *et al*. "Activation of C-C motif chemokine receptor 2 modulates testicular macrophages number, steroidogenesis, and spermatogenesis progression". *Cell Tissue Research* 386.1 (2021): 173-190.

13. Lukyanenko YO., *et al*. "Production of 25-hydroxycholesterol by testicular macrophages and its effects on Leydig cells". *Biology Reproduction* 64.3 (2001): 790-796.

14. Novikova EG., *et al*. "Experimental study of the effectiveness of rectal suppositories with oxidized dextran in prostate hyperplasia". *Urology* 5 (2021): 59-61.