



**MORPHOFUNCTIONAL CHARACTERISTICS OF CORRECTION OF REPERFUSION  
CHANGES OF TESTICLES PARENCHYMA IN RATS WITH DOSED COMPRESSION  
OF FUNICULUS**

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**ABSTRACT**

Due to the recent data of European Society of Human Reproduction and Embryology (ESHRE), about 1 million of couples are suffering from sterility in Ukraine, and that is 15-17 %. In the structure of causes of this pathology, 30% falls to the male factor, and this percentage is increasing. The disorders of reproductive function occur very often due to hemodynamic disorders which are considered to be one of the most common pathogenic factors in male infertility development. The experiments have been carried out on 78 non-linear white female rats. All the animals were divided into four groups: one control group and three experimental ones. At early stages of the experiment by the histological examination of the organ the moderate swelling of the interstitial tissue, which was unevenly distributed among sinuous canaliculi, was observed. Later, the disclosed changes continued to grow, and at the same time the number of the canaliculi in the line of vision was decreasing. In some sinuous seminal tubules the scaling of the spermatogenous epithelium from its own membrane was observed. On the 14<sup>th</sup> day of the experiment during the performance of the histological examination from the side of the vascular channel the moderate arterial plethora was observed only locally. The arteries had an ordinary look, their inner elastic membranes were mainly smooth. Sex cells were not rejected from the supporting ones, their layer could have been distinguished. In the lumen of the canaliculi there were matured spermatozoa.

**KEYWORDS:** testicle, compression, artery, circulation.

**INTRODUCTION**

It is a widely known fact that infertility in a marriage is a serious medical and biological, social and demographic problem. Equally, male and female factors may lead to it. Due to the recent data of European Society of Human Reproduction and Embryology (ESHRE), about 1 million of couples are suffering from sterility in Ukraine, and that is 15-17 %. In the structure of causes of this pathology, 30% falls to the male factor, and this percentage is increasing.<sup>[1,5,11]</sup> Such diseases as varicose veins of funiculus and testicle (9-40 %), hernia (0,8 %) or carried out inguinal hernioplasty (9,8 %) take significant place among the cases which lead to the disturbance of reproductive function in males. In males after herniotomia the disturbance of spermatogenic function is also marked from 1 to 50 %. Due to other data, herniotomia and inguinal hernioplasty are objectively considered as a factor of male sterility in 9,8 % of cases.<sup>[7]</sup> Inguinal hernia itself in 0,8 % may lead to

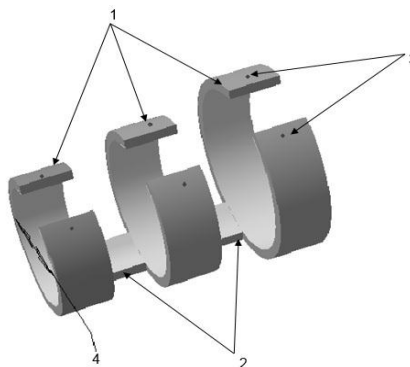
male infertility. By given pathologies the compression of funiculus is taking place, to some extent accompanied by the blood circulation disturbance in its vessels (the lesion of drainage systems of the testicle is observed). Along with this, the disorders of reproductive function occur very often due to hemodynamic disorders which are considered to be one of the most common pathogenic factors in male infertility development.<sup>[3,4,12]</sup>

**MATERIALS AND METHODS**

The experiments have been carried out on 78 non-linear white female rats, weighting 180-200 gramms which were fed due to a standard diet of vivarium. All the animals were divided into four groups: one control group and three experimental ones. The control group included six intact animals. All the other comprised the experimental group which was divided into three series six animals per each depending on the terms of observation. The animals with the model of dosed

stenosis of funiculus comprised first experimental group; the animals with the model of dosed stenosis and one-moment decompression, starting from the third day of the experiment comprised the second group; to the third group we referred the animals with the model of dosed stenosis of the funiculus which have undergone decompression with the correction of reperfusion changes due to the method proposed by us, starting from the third day.

The animals of the experimental group under the thiopental and natrium anesthesia ( $40 \text{ mgr} \times \text{kg}^{-1}$  of the body weight) have undergone the narrowing of the funiculus in  $1/3$  of its diameter by suturing. During this, the level of narrowing was being regulated with the help of metal probe with cone-shaped point.<sup>[8]</sup>



Rings of different diameters – 1, common base – 2, unlocked ends with the openings for ligature threads – 3, cavity of the rings – 4.

**Figure 1: The disassembled device.**

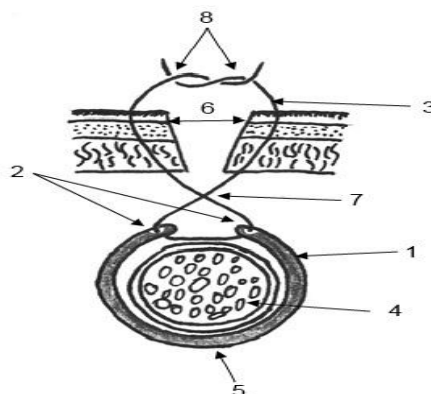
In order to correct reperfusion changes in the animals of the third experimental group, modeling of funiculus compression was being carried out with the help of the proposed device,<sup>[9]</sup> which consists of "Fig. 1" calibrating elements in the form of three lamellate rings of different diameter with unclosed ends on which the openings for the ligature has been done.

After the operative access to the funiculus has been performed "Fig. 2", it is placed into the device for the common base of the rings to be situated on the opposite to the operating wound wall. Ligatures are crossed and the opposite ends of the operational wound are sewed with them. From the third day of the experiment, every 24 hours one of the ligatures was taken off, starting with the ring with the smallest diameter of the opening. Such

a procedure has allowed performing decompression gradually and smoothly.

The rats were taken out of the experiment by means of total bloodletting from the heart under thiopental and natrium anesthesia ( $60 \text{ mg per kg}^{-1}$  of the body internally abdominal weight). All the experimental research was being performed in accordance with "Principles of Laboratory Animal Care".

On 1<sup>st</sup>, 3<sup>d</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of stenosis, the parts of testicles tissues were taken and fixed in 10 % neutral formalin solution, 96° spirit. Paraffin sections with 5-7 mcm were stained by hematoxylin and eosin, due to Weigert and Van Gieson.



Unclosed ring – 1, opening for the ligature thread – 2, ligature thread – 3, funiculus – 4, base of the rings – 5, operational cut – 6, ligature crossing – 7, bound ligature – 8.

**Figure 2: The funiculus of the rat, narrowed with the help of the proposed device.**

Morphometric evaluation of intraorganic vessels was performed with the help of ocular micrometer MOV-1-15CH (MOB-1-15Ч). The examination of the functional condition of the vessels was being carried out by calculating of the Index by Vohenvort (IV),<sup>[2]</sup> that is the ratio of the area of the middle artery's layer to the area of their lumen:

$$IV = (SM / SL) 100 \%,$$

For the morphometric study of the internally organic branching out the testicle arteries due to the caliber and topographic location were divided into three groups: the arteries of the protein membrane (AP) with the external diameter of 136-180 mcm, intramural arteries of the middle diameter (MD) with the external diameter of 51-135 mcm and small intramural arteries (SIA) with the external diameter of 26-50 mcm. Due to their morphometric characteristics these arteries fall to already known schemas of vascular channel gradation.<sup>[6,10]</sup>

Electronic materials received due to the carried out morphologic and functional research have undergone the statistic processing with the help of Microsoft Exel for Windows 98 taking into consideration average values and their standard deviations.

## RESULTS

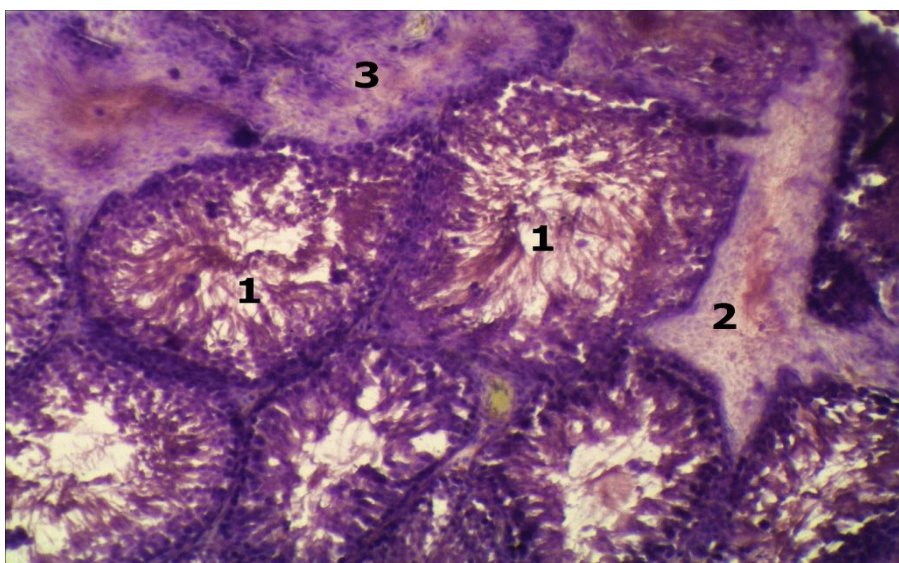
As a result of the research, it was defined that stenosing of the funiculus leads to the marked venous engorgement in the circulatory channel of the testis which is accompanied at early stages by reflex tonus increase and narrowing of the opening of small diameter intramural arteries with the compensatory widening of the arteries of middle diameter along with the increase of volume of the latter as well as of the arteries of the protein membrane. In the long run, it was typical to observe the development of progressive rising vasoconstriction with the reduced ability on all levels of the vessels to pass

blood.

The observed reactions of the circulatory channel of the testis were accompanied by the change in the structuring elements of testis parenchyma. At early stages of the experiment by the histological examination of the organ the moderate swelling of the interstitial tissue, which was unevenly distributed among sinuous canaliculi, was observed. Later, the disclosed changes continued to grow, and at the same time the number of the canaliculi in the line of vision was decreasing. In some sinuous seminal tubules the scaling of the spermatogenous epithelium from its own membrane was observed.

In the distant terms of the experiment the considerable accumulation of the given changes was observed. Besides, the focal haemorrhage with the disturbance of the completeness of internally organic vessels of the testis was noticed. As well one could observe clotting mainly occurring at the walls. The form of the sinuous seminal canaliculi was changing, their basal membrane was becoming thicker. In some places the spermatogenesis stopped on the level of spermatogonia, while more mature forms of the cells were absent. The replacement of testis parenchyma to connective tissue with formation of focus and even field of sclerosis was observed "Fig. 3". The number of sinuous canaliculi in the field of vision was decreasing considerably, partly at the expense of the increase in connective tissue elements.

On the seventh day of the experiment in animals with stenosis of the seminal funiculus, the thickness of the media with the narrowing of arteries opening was considerably growing. In addition to that the Vohenvort index in the middle intramural arteries exceeded control data in 7,24 %, and in 23,02 % in tiny ones "Fig. 4,5". In the arteries of the protein membrane this index was changing inconsiderably.

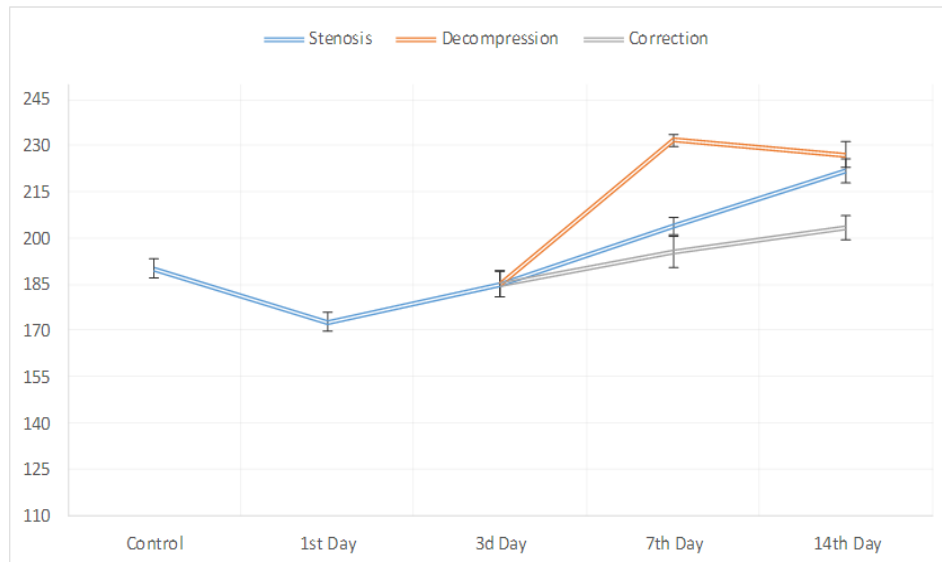


The opening of the canaliculus – 1, fibrosing of the seminal canaliculi – 2, homogenising zone – 3.

**Figure 3 – Histological section of rat's testis in 14 days after the stenosis of funiculus. Hematoxylin and eosin stain x 140.**

During the one-moment decompression of the seminal funiculus the given changes have become more expressed. In particular, in the middle and intramural tiny arteries the Vohenvort index was exceeding the control data in 21,09 % and 29,4 % respectively, and the

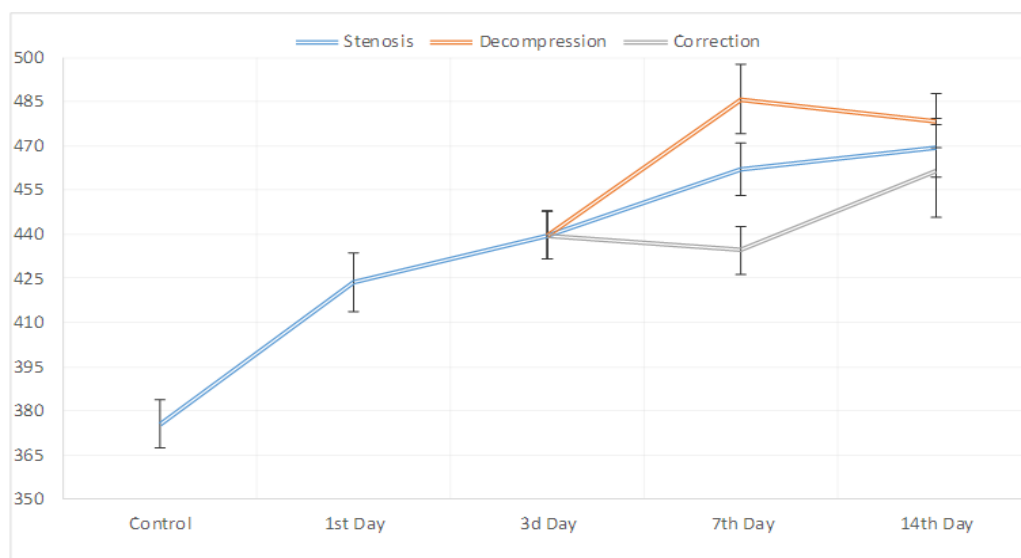
given index reliably decreased in relation to the norm in 9% in arteries of the protein membrane, and that avouches the increase of their volume and considerable disturbance of the hemodynamics of the organ "Fig. 6".



**Figure 4 – Dynamics of Vohenvort index in the arteries of the middle diameter.**

The use of the proposed device for the dosed narrowing of the diameter of the tubular biological objects had a positive impact on the peculiarities of rat's testis tissue restructuring due to the dosed decompression of the seminal funiculus. In animals which experienced correction, Vohenvort indices were changing in lesser extent. In particular, on the seventh day of the

experiment (the fourth day of compression) in the arteries of the protein membrane and in intramural arteries of the middle caliber it was approaching the indices of the control group "Fig. 4,6". At the same time, Vohenvort index of the middle caliber arteries was 18,5 % lesser than the corresponding index at one-moment decompression of the seminal cord "Fig. 5".



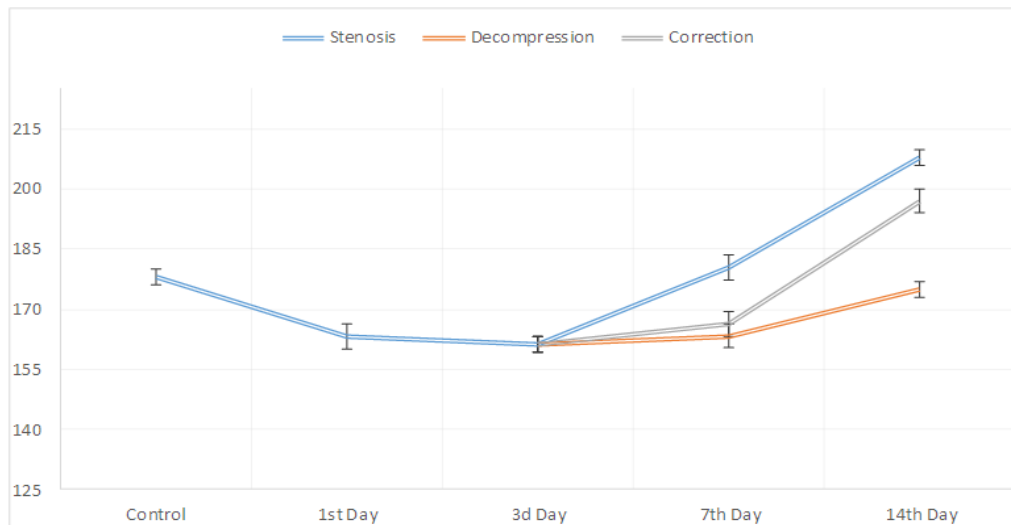
**Figure 5 – Dynamics of Vohenvort index in arteries of the tiny diameter.**

As to the tiny intermural arteries, the Vohenvort index remained higher than indices of the norm in 15,7%, but at the same time its reliable reduction in contrast to the

similar index at one-moment decompression of the seminal cord in 11,8 %.

On the 14<sup>th</sup> day of the experiment (the 11<sup>th</sup> day of the correction) at one-moment decompression of the seminal cord the increase of the index by Vohenvort and the

thickening of the media in arteries of all orders under examination was observed.



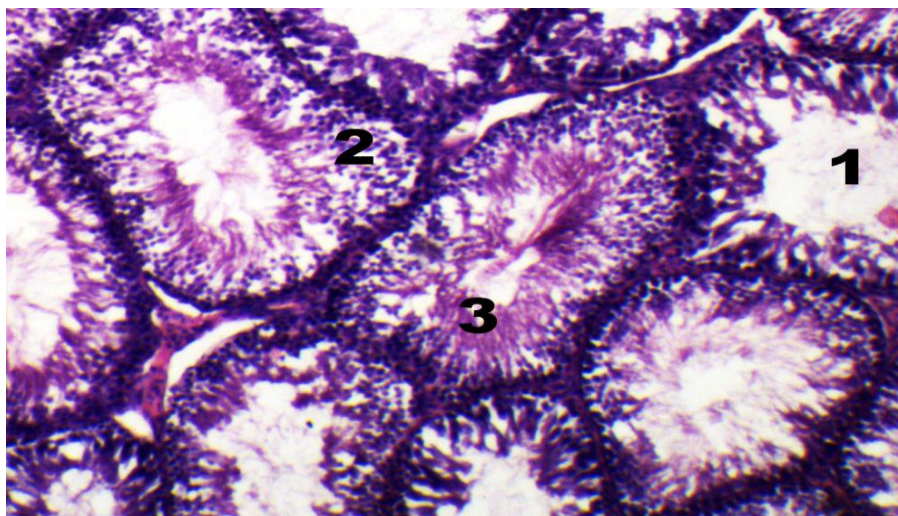
**Figure 6 – Dynamics of the index by Vohenvort in the arteries of big diameter.**

Comparing the control numbers it was veraciously increasing in middle and tiny intramural arteries in 19,4 % and 27,4 % respectively. Using the proposed device the growth of the index, in particular in the arteries of the protein membrane in 10,6 % and in tiny arteries in 22,9 % was seen, especially if to compare it with the indices of the control group. However, the increase of the index by Vohenvort in intramural arteries of the middle caliber was veraciously lesser in 11,7 % than at one-moment decompression.

During the performance of the histological examination at the given period of time from the side of the vascular channel "Fig. 7" the moderate arterial plethora was observed only locally.

The arteries had an ordinary look, their inner elastic membranes were mainly smooth. Sex cells were not rejected from the supporting ones, their layer could have been distinguished. In the lumen of the canaliculi there were matured spermatozoa.

Thus, on the basis of received results during the experimental research we may assume, that the application of the proposed device for the narrowing of the tubular biological objects and their dosed decompression in order to correct reperfusion changes allows to receive positive changes from the side of the vascular channel and organ's parenchyma.



The lumen of the canaliculus – 1, the spermatogenous epithelium cells – 2, plait-like spermatozoon ropes in the lumen of the canaliculus – 3.

**Figure 7 – Histological cut of the rat's testis on the 14<sup>th</sup> day (the 11<sup>th</sup> day of correction) after the correction of reperfusion changes. Hematoxylin and eosin stain x 140.**

## CONCLUSIONS

The use of the device for diameter narrowing of the tubular biological objects prevents appearing of reperfusion changes in the rat's testis, making the blood circulation better which helps to preserve integrity of the seminal canaliculi and the process of spermatogenesis in them.

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