

## **EXPERIMENTAL STUDY OF THE SENSITIVITY OF SPERMATOGENIC EPITHELIAL CELLS TO SHORT-TERM ISCHEMIA**

**Tairov Doston Rustamovich, PhD, Assistant  
Department of Propaedeutics of Internal Diseases  
Samarkand State Medical University**

**Abstract:** In experimental conditions, short-term ischemia was induced in white rats by applying a soft clamp to the vas deferens, after which the cells of the spermatogenic epithelium were studied. After a certain period of time, the obtained material was fixed in Bouin's solution. Staining was performed with hematoxylin-eosin and with hematoxylin using the Schiff-Ehrlich method. The cells of the spermatogenic epithelium were examined at stage VII of the spermatogenic cycle.

**Keywords:** experiment, white rat, ischemia, hematoxylin-eosin method, Schiff's reagent, hematoxylin by Ehrlich's method, spermatogenic epithelial cells.

## **ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ ЧУВСТВИТЕЛЬНОСТИ КЛЕТОК СПЕРМАТОГЕННОГО ЭПИТЕЛИЯ К КРАТКОВРЕМЕННОЙ ИШЕМИИ**

**Тайров Достон Рустамович, PhD, ассистент  
Кафедра пропедевтики внутренних болезней  
Самаркандский государственный медицинский университет**

**Аннотация:** В экспериментальных условиях у белых крыс путём наложения мягкого зажима на семявыносящий проток вызывалась кратковременная ишемия, после чего изучались клетки сперматогенного эпителия. Через определённый период времени полученный материал фиксировался в растворе Буэна. Окраска проводилась гематоксилином-эозином и по методу Шиффа-Эрлиха гематоксилином. Клетки сперматогенного эпителия исследовались на VII стадии цикла сперматогенеза.

**Ключевые слова:** эксперимент, белая крыса, ишемия, метод гематоксилином-эозином, раствор Шиффа, гематоксилином по методу Эрлиха, клетки сперматогенного эпителия.

**Introduction.** In recent years, interest in the problem of male infertility has significantly increased. It is becoming increasingly evident that one of the causes leading to the development of infertility is varicocele. Modern scientists assign one of the leading places to the diagnosis and treatment of varicocele in the problem of infertility [1,2]. Male infertility is a condition that results from a number of diseases and/or cumulative pathological effects on the male reproductive system [3,4,5]. The most common andrological pathology and the most frequent cause of male infertility is varicocele (varicose dilatation of the pampiniform plexus veins of the spermatic cord) [6,7]. As is known, the spermatogenic epithelium exhibits high sensitivity to hypoxia. However, the question of the selective effect of this factor on germ cells at different stages of development has been insufficiently studied. In the present work, we investigated the sensitivity of germ cells at different degrees of differentiation to the action of temporary ischemia.

**Aim of the study.** We studied the experimental sensitivity of spermatogenic epithelium cells to short-term ischemia.

**Materials and methods.** The experiments were performed on 22 white outbred male rats weighing 180–230 g. The testes were subjected to 3, 5, 10, 15, and 30-minute ischemia by applying a soft clamp to their blood vessels. The resulting pathological changes in testicular tissue were studied at 1, 7, 30, and 90 days after restoration of blood circulation. The material was fixed in Bouin's fluid or Zenker-formol. Paraffin sections 6–7  $\mu\text{m}$  thick were stained with hematoxylin-eosin and Schiff's reagent with counterstaining with Ehrlich's hematoxylin. The number of spermatogenic cells at stage VII of the spermatogenic epithelium cycle was determined. The selective sensitivity of germ cells to temporary ischemia was judged by changes in the number of type A spermatogonia, preleptotene spermatocytes, pachytene spermatocytes, and step 7 spermatids. The obtained results were recalculated per 100 Sertoli cells.

**Results.** Cell counts were performed in 40 seminiferous tubules for each case at  $\times 900$  magnification. It was found that 24 hours after 30-minute ischemia, the

number of type A spermatogonia was  $8.97 \pm 1.03$  and remained unchanged compared to control animals. A statistically significant (by 33.11%) decrease in the number of these cells was observed only 30 days after 30-minute ischemia. At 1, 7, 30, and 90 days after 30-minute ischemia, the number of preleptotene spermatocytes did not change. 5–10-minute ischemia was accompanied by a slight (by 6%) decrease in the number of these cells; however, by 7 days their number gradually normalized and reached the initial level by 30 days. 15-minute ischemia was accompanied by deformation of some seminiferous tubules, progressive decrease in preleptotene spermatocytes with destructive changes, necrosis, and disintegration. The number of cells at 30 days was  $202.62 \pm 4.13$  compared to  $230.58 \pm 2.52$  in the testes of control animals. 30-minute ischemia caused a more significant (34.70%) reduction in the number of these cells, which were completely absent in atrophied seminiferous tubules by 90 days. The number of pachytene spermatocytes in the seminiferous tubules of control animals was  $299.82 \pm 4.43$ . 3–5-minute ischemia had no pronounced effect on the quantitative indicators of this generation of cells throughout all periods. No normalization of their number was observed during the entire experiment. A statistically significant decrease in the number of these cells occurred under 15-minute ischemia, amounting to  $246.85 \pm 2.6$  by the 90th day of the experiment. 30-minute ischemia, compared to other durations, had the most substantial effect on this cell type: their nuclei were pyknotic, cytoplasm vacuolated, with transformation into cellular detritus in some tubules, and their number decreased to  $97.79 \pm 11.97$  by 30 days. Among the spermatogenic cells of the seminiferous tubules, the most numerous ( $916.76 \pm 22.06$ ) are spermatids. 3-minute ischemia throughout the experiment (1, 7, 30, 90 days) did not cause a statistically significant decrease in the number of spermatids. However, already 5-minute cessation of blood flow reduced their number to  $838.61 \pm 11.91$  by 7 days; by 90 days of the experiment, their number was  $895.37 \pm 32.32$ . Temporary 10-minute ischemia was accompanied by a noticeable decrease in the number of these cells during the first week. Then their number increased but did

not reach the initial values. Step 7 spermatids responded more sharply to 15-minute ischemia: nuclei deformed, cytoplasm unevenly stained, their number decreased to  $816.26 \pm 11.05$  by the weekly term of the experiment, and to  $736.57 \pm 7.89$  by 30 days. 30-minute testicular ischemia by 30 days of the experiment led to destruction and disintegration of this cell type with a sharp decrease in their number, followed by complete depletion of the seminiferous tubules. Thus, it was established that, despite the identical conditions of the experiments, pathological changes in the testes of different animals within the same group were heterogeneous in nature.

**Conclusion.** The results of the present work indicate the vulnerability of all types of germ cells after 30-minute temporary ischemia. The most vulnerable among them were spermatocytes at different stages of meiotic prophase. At the same time, the possibility of recovery processes in the seminiferous tubules under conditions of 10-minute blood flow interruption, the beginning of which was noted for spermatocytes and step 7 spermatids on days 30 and 90 of the experiment, suggests the resistance of part of type A spermatogonia to temporary ischemia of the specified duration. The preserved spermatogonia serve as a source for the subsequent development of germ cells in accordance with the duration of individual stages of their differentiation.

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