Impairment of Spermatogenesis in Male Rats during Stress

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Stress is followed by severe disturbances in maturation of male gametes in the testicles, which concerns all populations of cells in the spermatogenic epithelium and modulates the function of endocrine cells (Leydig cells). The observed changes result in reproductive dysfunction of male albino rats.

Key Words: *stress; spermatogenesis; spermatozoa*

The incidence and severity of various diseases related to adverse effects of ecological and social environmental factors (*e.g.*, stress) are constantly increasing [1,5,6]. The male reproductive system is most susceptible in this respect. The mean concentration and total content of sperm cells in European men decrease by 2% per year. The majority of investigators believe that the decrease in human spermatogenic function is an objective process. This process is associated with the influence of environmental factors and stress [3,9,10]. The effect of stress on spermatogenesis is difficult to be studied. In clinical practice, it is difficult to evaluate the stress component of disturbances in gamete maturation. Several clinical trials revealed a direct correlation between sterility of married couples and psychoemotional strain under stress conditions. For example, impairment of spermatogenesis and menstrual cycle was more often observed in victims of the Armenian earthquake (compared to other Armenians). For example, blood testosterone concentration sharply decreased in men. Little is known about the role of stress in sex dysfunction. The majority of studies concern female reproductive function [2].

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This work was designed to study reproductive dysfunction in male albino rats during acute experimental stress.

MATERIALS AND METHODS

Experiments were performed on 39 adult male outbred albino rats weighing 250-300 g. Immobilization stress (IS) was induced by the method of H. Selye. The animals were immobilized in the supine position for 6 h (day 1 of the study) [8]. Intact males served as the control. Sperm number in the ejaculate was estimated on days 1, 7, 14, 30, and 60. The ejaculate was obtained by electrical stimulation of the seminal colliculus through the rectal mucosa [7]. The number and motility of spermatozoa were evaluated in a Goryaev chamber. A cytological study was performed with stained smears of testicular homogenates. The spermogram was calculated as the percent of various cells in the spermatogenic epithelium (spermatogonia, sperm cells, early and late spermatids, and spermatozoa), Sertoli cells, and Leydig cells. Germinal epithelial cells, supporting cells, and endocrine cells in the testicles were also counted. The following integral coefficients were measured: maturation index, germinative index, meiotic index, and index of spermatogenesis [4].

The results were analyzed by means of Statistica 6.0 software. The significance of differences was evaluated by Student's t test ($p<0.05$).

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RESULTS

Studying the seminal fluid from treated males showed that the number of spermatozoa in the ejaculate decreases 1 day after IS (2.79±0.23 million *vs*. 11.70±0.54 million cells in the control). Motile cells were not revealed in stressed animals (84.1±2.0% motile gametes in the control). One week after stress, the number of sperm cells was 0.02±0.01 million. Motile cells were not detected in this period. Similar changes were observed in the follow-up period. The number of spermatozoa in the ejaculate slightly increased by the end of the 2nd month after IS (up to 0.12±0.01 million). Motile gametes were not found.

Examination of the testicular tissue revealed progressive changes in quantitative and qualitative parameters of spermatogenesis after single exposure to IS. The amount of various cells of the spermatogenic epithelium significantly decreased 1 week after stress (Fig. 1). The maturation index increased to 0.56±0.10, which reflected a decrease in the number of mature cells in the testicular tissue (early and late spermatids and spermatozoa; Table 1). The meiotic index decreased by 1/3 and corresponded to 0.16±0.01. Decreasing the meiotic index serves as a sign for delayed differentiation of germ cells from spermatids into spermatozoa. The observed changes reflect an indirect decrease in total cell number in the testicle and epididymis. The 5-fold decrease in the germinative index (ratio of germinal epithelial cells to supporting cells) illustrates the prevalence of Sertoli cells and significant reduction of spermatogonia in the gland. These changes are prognostically unfavorable for the recovery of spermatogenesis. The relaxation index (index of spermatogenesis) decreased to 1.75±0.23. Specific productivity of the testicles decreased after 1 week. The changes were most significant in populations of mature cells.

Figure 1 shows the number of endocrine cells in testicular tissue (Leydig cells). The number of these cells decreased by 2 times 1 week after IS

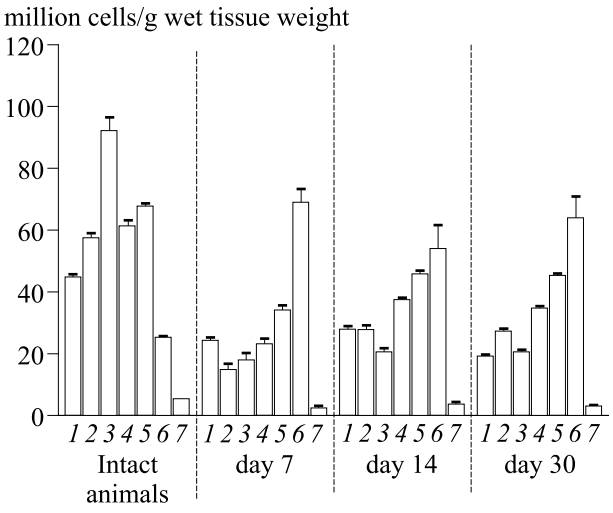

Fig. 1. Number of spermatogenic epithelial cells, Sertoli cells, and Leydig cells in the testicles after IS. Spermatogonia (*1*), sperm cells (*2*), early spermatids (*3*), late spermatids (*4*), spermatozoa (*5*), Sertoli cells (*6*), and Leydig cells (*7*).

(from 5.36±0.07 to 2.47±0.68 million cells/g tissue). These changes probably result in inhibition of spermatogenesis due to a decrease in testosterone concentration. Single exposure to 6-h immobilization was followed by a significant decrease in testosterone concentration (by 44%) and increase in plasma progesterone concentration in rats (by 39%). These changes persisted for 3 days after IS. The increase in the concentrations of lactate and pyruvate in testicular tissue reflects progressive hypoxia of the gonads.

The impairment of spermatogenesis became more severe 2 weeks after stress exposure. The number of spermatozoa, early spermatids, and late spermatids in the testicles was 45.86±1.36, 20.62±1.81, and 37.44±3.11 million cells/g tissue, respectively. The number of sperm cells remained low (7.83±1.45 million cells/g tissue). The meiotic index returned to normal due to simultaneous decrease in all populations of the germinal epithelium. The maturation index was 0.48±0.12. The germinative index (0.59±0.09) was 3-fold lower than in the control. The index of spermatogenesis was 4 times

TABLE 1. Integral Parameters of Spermatogenesis in Male Albino Rats during IS

Parameter	Normal	Experimental IS		
		after 7 days	after 14 days	after 30 days
Maturation index	0.43±0.02	0.56±0.10	0.48±1.20	0.45±0.02
Meiotic index	0.21±0.02	0.16±0.01	0.21±0.01	0.22±0.20
Germinative index	1.76±0.18	0.34±0.12*	0.53±0.09*	0.31±0.01*
Index of spermatogenesis	12.77±0.10	1.75±0.11*	3.37±0.10*	2.51±0.17*

Note. *$p<0.05$ compared to normal.

lower compared to the control. Two weeks after stress exposure, this parameter was 3.37±0.10. These changes reflect a progressive decrease in the total number of germ cells in the testicles.

The number of germinal epithelial cells progressively decreased in the testicles. The supporting Sertoli cells prevailed under these conditions. Hence, single stress exposure is followed by severe and persistent dysfunction in maturation of male gametes. Pathological changes were observed in all generations of germ cells and Leydig cells (endocrine cells), which increases the severity of spermatogenesis impairment. Our findings confirm the hypothesis that stress is one of the triggering mechanisms for male reproductive dysfunction. Parameters of gametogenesis can serve as a reliable and early criterion for adaptation and disadaptation to adverse factors.

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