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The effect of major salivary gland hypertrophy on rat's spermatogenic epithelium ultrastructure of rats

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ABSTRACT

Purpose. The aim of this study was to ascertain the characteristics of major salivary glands endocrine effect on spermatogenesis.

Materials and methods. Mature white outbred male rats (2 months, 153 ± 18 g) consisted of the following groups (each containing 30 rats): intact, control, and group of rats subjected to multiple amputation of incisors. To achieve hypertrophy of major salivary glands multiple amputation of incisors was performed: incisors were cut to a level of 1-2 mm above the gingival margin under ether anesthesia once every 3 days within 2 weeks. Animals of the control group were anesthetized with ether at the same time. Rats were sacrificed by CO2 asphyxia after 2, 3, 4, 6, 8 and 10 weeks after the first amputation of incisors. Fragments of the rat testes were examined on a JEM-1400 "JEOL" (Japan) transmission electron microscope. On electron microscopy images the specific vacuolization of the cytoplasm of Sertoli cells, spermatogonia, spermatocytes and spermatids (standard units) was analyzed by the point counting method. In spermatogenic cells the proportion of mitochondria (%) with morphological signs of swelling was assessed.

Results. Transient ultrastructural changes of Sertoli and spermatogenic cells develop in the rats convoluted seminiferous tubules as a result of multiple amputation of the incisors, such as phagosomes and pronounced vacuolization in the Sertoli cells cytoplasm, cytoplasm vacuolization and mitochondrial swelling in spermatogenic cells. Sporadic spermatogenic cells with signs of nuclear (chromatin fragmentation, its condensation on the periphery of the nucleus) and cytoplasm (destruction of membrane organelles) destruction appeared as a result of multiple incisors' amputation. Ultrastructural changes of Sertoli and spermatogenic cells are most pronounced at 2-3 weeks, decrease at 4 week and are completely leveled by the 6th week of the experiment.

Conclusion. Hypertrophy of major salivary glands, caused by multiple amputations of incisors, has similar to sialoadenectomy effect on the spermatogenic epithelium. Multiple incisors' amputation cause transient depression of granular convoluted cells function. Probably submandibular gland granular convoluted tubules cells endocrine factors make the greatest contribution to the regulation of spermatogenesis in rats.

Key words: spermatogenesis, Sertoli cells, salivary glands, hypertrophy.

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Влияние гипертрофии больших слюнных желез на ультраструктуру сперматогенного эпителия крыс

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РЕЗЮМЕ

Цель. Выяснение особенностей эндокринного влияния больших слюнных желез на сперматогенез половозрелых крыс.

Материалы и методы. Половозрелые белые беспородные самцы крыс (возраст 2 мес, масса тела (153 ± 18) г) составили три группы (по 30 особей): интактная, контрольная и крысы, подвергшиеся многократной ампутации резцов. Для оценки эндокринного влияния эпителиоцитов ацинусов и протоков больших слюнных желез моделировали их гипертрофию путем многократной ампутации резцов. Крыс выводили из эксперимента на 2-, 3-, 4-, 6-, 8- и 10-ю нед после первой ампутации резцов. Семенники животных оценивали при помощи трансмиссионной электронной микроскопии. На электронограммах анализировали удельный объем вакуолизации цитоплазмы сустентоцитов, сперматогоний, сперматоцитов и сперматид (усл. ед.), в сперматогенных клетках оценивали количество митохондрий (%) с морфологическими признаками набухания.

Результаты. В ранние сроки эксперимента в извитых семенных канальцах крыс развивается вакуолизация цитоплазмы сустентоцитов, а также ультраструктурные изменения сперматогенных клеток (вакуолизация цитоплазмы, альтерация митохондрий, разрушение мембранных органелл, фрагментация хроматина). Изменения структуры сперматогенных клеток и сустентоцитов максимально выражен на 2–3-й нед, снижаются на 4-й нед и полностью нивелируются к 6-й нед эксперимента.

Заключение. Гипертрофия больших слюнных желез, вызванная многократной ампутацией резцов, оказывает на сперматогенный эпителий влияние, схожее с эффектом сиалоаденэктомии. В результате многократной ампутации резцов угнетается функциональное состояние клеток гранулярных извитых трубок поднижнечелюстных желез. Эндокринные факторы, которые вносят наибольший вклад в регуляцию сперматогенеза у крыс, вырабатываются клетками гранулярных извитых трубок.

Ключевые слова: сперматогенез, сустентоцит, слюнные железы, гипертрофия.

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INTRODUCTION

The major salivary glands are duacrine glands [1]: their exocrine function is associated with digestion, taste reception, non-specific immune defense, excretion and speech production. They have a proven endocrine effect on the organs of the hematopoietic and immune systems, skin, nephron epithelial cells, cartilage, and the gonads [2, 3]. The mutual influence of the salivary glands and gonads is present in many animals, including humans. It has been proved that a complete medical examination makes it possible to diagnose the interstitial form of sialadenosis (sialosis) in 100% of both male and female patients with hypogonadism [4]. Rodents are the most convenient model

for studying the mutual influence of the gonads and salivary glands because of the pronounced morphological and biochemical sexual dimorphism of their major salivary glands. Thereby, the aim of this study was to elucidate the features of the endocrine effect that the major salivary glands have on spermatogenesis of rats.

MATERIALS AND METHODS

Mature, white, outbred male rats (age 2 months, body weight 153 ± 18 g) constituted the following groups (each containing 30 rats): intact (I), control (C), and the group of rats subjected to repeated amputation of incisors (RA). The hypertrophy for RA rats was simulated through repeated amputation of incisors. Lower and upper incisors were trimmed under ether anesthesia to a level of 1–2 mm above the gingival margin once every 3 days within 2 weeks (5 amputations in total). Animals of the control group were narcotized with diethyl ether with the same periodicity. Removal from the experiment was carried out by CO2 asphyxiation during the 2nd, 3rd, 4th, 6th, 8th and 10th weeks after the first amputation of incisors.

Fragments of the rat testes were fixed for 24 hours in 4% paraformaldehyde (Serva, Germany), then for 3 hours in 1% OsO4 (SPI, USA) at 4 ° C, pH 7.4. The samples were immersed in a mixture of epoxy resins Epon 812: Araldite 502: DDSA (SPI, USA). Ultrathin sections (80 nm) were obtained on an ultratome (eica EM UC 7 (Leica, Austria) and contrasted with uranyl acetate and lead citrate for examination with a JEM-1400 transmission electron microscope (JEOL, Japan).

On electron diffraction patterns, the specific volumes of cytoplasmic vacuolization of Sertoli cells, spermatogonia, spermatocytes, and spermatids (standard units) were analyzed using the program ImageJ 1.48 (NIH Image, USA). In spermatogonia, spermatocytes, and spermatids the number of mitochondria (%) with morphological signs of swelling was assessed (calculations were based on the analysis of 200 mitochondria).

Statistical processing of quantitative data was performed using the Shapiro – Wilk, Mann – Whitney, and Kruskal – Wallis tests and SPSS 17.0 (IBM, USA). The results of the morphometric study are presented as the median and interquartile range *Me* (Q_1; Q_3), the significance level is taken as $p < 0.05$.

RESULTS

In the convoluted seminiferous tubules of rats of the intact (I) and control (C) groups, Sertoli cells and all populations of germ cells were determined throughout the period of study. However, in animals of the RA group, spermatozoa were found in the lumens of the convoluted seminiferous tubules starting from the 3rd week of the experiment. During the study period, the rats of the C group showed no difference in the ultrastructure of Sertoli and spermatogenic cells from those of the group I (Tables 1, 2).

Table 1

The specific volume of cytoplasmic vacuolization, standard units, *Me* (Q_1; Q_3)

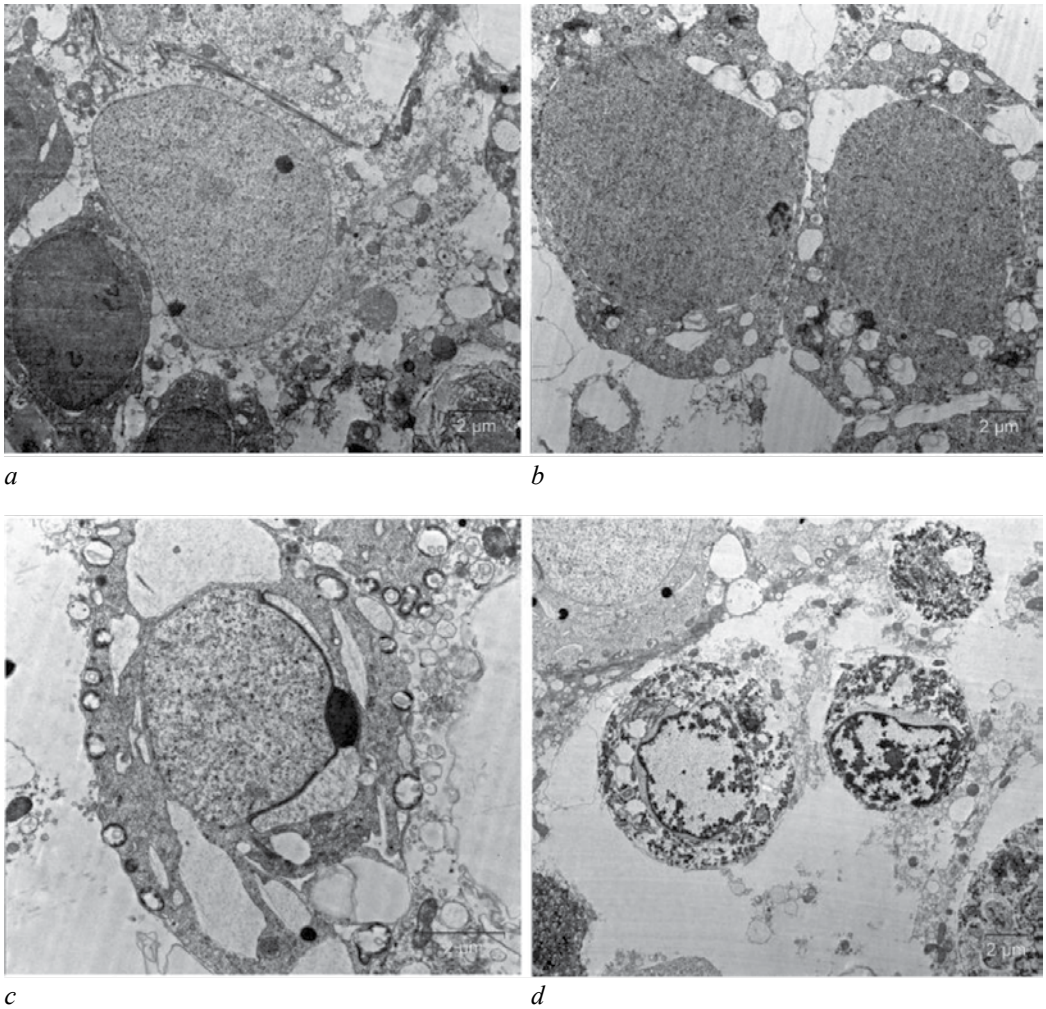
Experiment duration, week	Sertoli cells			Spermatogonia			Spermatocytes			Spermatids		
	I	C	RA	I	C	RA	I	C	RA	I	C	RA
2nd	0 (0; 1.5)	0 (0; 1.2)	36.8 (24.7; 45.0)*	0 (0; 4.8)	0 (0; 3.6)	9.9 (5.6; 18.1)*	0 (0; 4.2)	0 (0; 1.6)	10.2 (7.1; 14.4)*	0 (0; 1.6)	0 (0; 2.1)	10.0 (7.3; 25.9)*
3rd	0 (0; 2.1)	0 (0; 2.6)	37.9 (14.5; 44.2)*	0 (0; 1.6)	0 (0; 4.5)	4.2 (2.4; 9.7)	0 (0; 2.6)	0 (0; 2.0)	6.9 (0.9; 9.9)	0 (0; 2.5)	0 (0; 1.0)	10.7 (5.1; 22.8)*
4th	0 (0; 1.2)	0 (0; 1.6)	16.1 (14.7; 21.6)*#	0 (0; 3.7)	0 (0; 0.6)	2.0 (0; 4.1)	0 (0; 1.4)	0 (0; 1.6)	3.8 (0; 16.0)	0 (0; 1.5)	0 (0; 2.1)	6.2 (1.0; 14.8)#
6th	0 (0; 0.8)	0 (0; 1.4)	0 (0; 6.7)#	0 (0; 0.7)	0 (0; 1.0)	0 (0; 1.6)	0 (0; 2.0)	0 (0; 0.7)	0 (0; 1.6)	0 (0; 0.9)	0 (0; 1.2)	0 (0; 3.1)
8th	0 (0; 0.6)	0 (0; 1.6)	0 (0; 1.1)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 1.3)	0 (0; 2.3)	0 (0; 0)	0 (0; 0)	0 (0; 1.6)
10th	0 (0; 1.0)	0 (0; 0.6)	0 (0; 0.8)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0.7)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)

Note. I – intact group, C – control group, RA – the group of rats subjected to repeated amputation of incisors (here and in Table 2).
*difference between the indicator and the corresponding indicator of the intact group, $p < 0.05$.
#difference between the present indicator and that during the previous period within the same group, $p < 0.05$ (here and in Table 2).

At 2–4 weeks of the experiment, phagosomes, phagolysosomes and cytoplasmic vacuolization were detected in the cytoplasm of the Sertoli cells in rats of the RA group (Fig., *a*). The observed vacuoles were dilated cisterns of the endoplasmic reticulum (EPR). The severity of vacuolization was maximal at 2–3 weeks, though decreased over time and completely leveled off by the 6th week of the experiment ($p < 0.05$; see Table 1). Vacuolization of the cytoplasm was observed in spermatogonia, spermatocytes (order I and II) in rats of the RA group during the 2nd week of the experiment ($p < 0.05$; see Table 1).

Mitochondrial swelling, which was identified as a decrease in the number and size of cristae, expansion of matrix and the appearance of vesicular structures in it [6], was observed in spermatogonia at 2–3 weeks, and in spermatocytes, at 2–4 weeks after the first amputation of incisors ($p < 0.05$; see Table 2; Fig., *b*). The ultrastructural changes in spermatogonia described above and developing in response to repeated amputation of incisors completely leveled off by the 4th week, and in spermatocytes, by the 6th week of the experiment.

At 2–4 weeks of the experiment, early and late spermatids in rats of the RA group were characterized by mitochondrial swelling ($p < 0.05$; see Table 2) and membrane destruction. Expansion of the EPR cisterns and the Golgi apparatus was also detected in early spermatids at 2–3 weeks ($p < 0.05$; Table 1; Fig., *b*). Structural changes in spermatids caused by repeated amputation of incisors leveled off by the 6th week of the experiment.



a *b*

c *d*

Figure. Fragment of the convoluted seminiferous tubule of a mature rat subjected to repeated amputation of incisors: *a* – cytoplasmic vacuolization of a Sertoli cell; *b* – cytoplasmic vacuolization, expansion of the perinuclear space and mitochondrial alteration in first-order spermatocytes; *c* – cytoplasmic vacuolization and destruction of mitochondrial membranes in an early spermatid; *d* – spermatogenic cells with signs of destruction of the nucleus and cytoplasm; transmission electron microscopy; 2nd week (*a*, *d*) and 4th week (*b*, *c*) of the experiment

Table 2

The number of mitochondria with signs of swelling, %, *Me* (Q_1; Q_3)

Experiment duration, week	Spermatogonia			Spermatocytes			Spermatids		
	I	C	RA	I	C	RA	I	C	RA
2nd	0 (0; 2.8)	0 (0; 2.5)	8.0 (5.0; 11.0)*	0 (0;2.4)	0 (0; 2.5)	25.6 (17.2; 39.2)*	0 (0; 4.1)	0 (0; 6.4)	36.8 (30.4; 47.0)*
3rd	0 (0; 2.5)	0 (0; 2.2)	10.0 (6.6; 11.9)*	0 (0; 2.1)	0 (0; 4.4)	32.0 (20.4; 37.3)*	1.4 (0; 5.3)	0 (0; 2.8)	31.8 (26.2; 39.1)*
4th	0 (0; 3.2)	0 (0; 1.3)	0 (0; 3.7)#	0 (0; 3.2)	0 (0; 4.0)	14.7 (6.0; 25.2)*#	0 (0; 7.1)	0 (0; 2.2)	24.5 14.1; 29.8)*#
6th	0 (0; 2.8)	0 (0; 3.1)	0 (0; 1.6)	0 (0; 1.9)	0 (0; 3.8)	4.0 (1.5; 8.9)#	0 (0; 3.1)	0 (0; 6.5)	6.0 (1.5; 10.3)#
8th	0 (0; 2.2)	0 (0; 2.0)	0 (0; 2.6)	0 (0; 1.5)	0 (0; 0.8)	0 (0; 4.6)	0 (0; 1.6)	0 (0; 2.0)	0 (0; 0.5)
10th	0 (0; 0.5)	0 (0; 1.0)	0 (0; 1.5)	0 (0; 0.5)	0 (0; 0.5)	0 (0; 0)	0 (0; 0.5)	0 (0; 0.7)	0 (0; 0)

At 2–3 weeks of the experiment, the spermatogenic epithelium of the RA group demonstrated individual germ cells with signs of nuclear destruction (fragmentation of chromatin, its condensation along the periphery of the nucleus) and cytoplasm (destruction of membrane organelles). Cells with signs of destruction are round in shape and have adluminal localization (see Fig. 1, d), which allows researchers to identify them as spermatocytes or early spermatids. Starting from the 4th week of the experiment, no spermatogenic cells with signs of destruction were detected in the convoluted seminiferous tubules of the RA group.

During the study period, we did not observe changes in the morphology of spermatozoa and peritubular myoid cells in rats in response to repeated amputation of incisors.

DISCUSSION

We have previously made a conclusion that the removal of the major salivary glands leads to ultrastructural changes in spermatogenic epithelium of immature rats [3]. However, it remains unclear which structures of the major salivary glands are the source of factors that have the greatest influence on testes. For example, sialorphin and parotin are produced by the acini of the submandibular and parotid glands respectively [7]. Epidermal growth factor, transforming growth factor α and β are produced by cells in the ducts of the submandibular glands [8]. All of the above and possibly some other biologically active factors of the major salivary glands affect spermatogenesis and steroidogenesis. Repeated amputation of incisors causes hypertrophy exclusively in epithelial cells of the acini in the major salivary glands, though it is not accompanied by hyperfunction [9]. On the contrary, repeated amputation of incisors inhibits the functional state of cells in the granular convoluted tubules of the submandibular glands [9]. Thus, the chosen experimental model will make it possible to assess the contribution of the acini and ducts of the major salivary glands to the endocrine regulation of spermatogenesis.

Cytoplasmic vacuolization of Sertoli cells is a non-specific response to damage [10] and indicates a violation of cell metabolism [11]. Sertoli cells are involved in the regulation of spermatogenesis in the paracrine way (inhibin, activin, anti-Müllerian hormone), as well as through contact with germ cells [12]. Sertoli cells are a labile element of the blood-testis barrier and take part in the formation of the microenvironment for developing germ cells [12]. Dysfunction of Sertoli cells inevitably leads to dysregulation of spermatogenesis.

Ultrastructural changes in spermatogenic cells that develop in response to repeated amputation of incisors affect the energy and synthetic apparatuses of cell. Swelling and destruction of mitochondria (2–4 weeks) in spermatogenic cells of the RA group indicate a decrease in the intensity of energy processes in them. Mitochondria in germ cells perform many functions: they are involved in the initiation of apoptosis in defective germ cells, ensure the motility of spermatozoa, and their controlled production of active radicals is necessary for proper capacitation and acrosome reaction [13]. Defects in the ultrastructure of mitochondria are associated with impaired sperm functioning [13]. Mitochondrial alteration is associated with excessive production of active radicals, which are potential inducers of cytoplasmic vacuolization. Damage to the membrane and enzyme systems of the granular EPR is the cause of the violation of protein folding and deg-

radation, which leads to the expansion of the lumen of its cisterns [14, 15].

Spermatids and spermatocytes are the most sensitive spermatogenic cells when it comes to changes caused by repeated amputation of incisors. In a number of spermatogenic cells, ultrastructural changes become irreversible. The presence of germ cells with signs of destruction (2–3 weeks) and the absence of spermatozoa (2 week) in the convoluted seminiferous tubules indicate the impossibility of a proper maturation phase and formation of spermatogenesis in rats shortly after reaching hypertrophy of the major salivary glands through repeated amputation of incisors. Phagolysosomes detected in the cytoplasm of Sertoli cells at 2–4 weeks of the experiment are likely the result of absorbing the fragments of destroyed spermatogenic cells.

The ultrastructural changes in Sertoli and spermatogenic cells observed in response to repeated amputation of incisors are similar to those developing after sialoadenectomy [3]. Since repeated amputation of incisors leads to the acini hypertrophy, the reduced number of cells in the ducts of the submandibular glands and their inhibited functional activity [9] suggests the following: it is the granular convoluted tubules of the submandibular glands that produce factors having the greatest effect on spermatogenic epithelium. The suppression of synthetic and secretory activity in cells of the granular convoluted tubules of the submandibular glands leads to the development of ultrastructural changes in cells of the convoluted seminiferous tubules. Epidermal growth factor, transforming growth factor α and β, and other biologically active factors of epithelial cells in the ducts of the submandibular glands can have a direct effect on germ cells or an effect mediated by Sertoli cells and interstitial endocrinocytes of testis. It is worth noting the potential endocrine action of the major salivary glands on spermatogenesis indirectly, through the central and peripheral endocrine glands. Biologically active substances of the granular convoluted tubules of the submandibular glands in rats are also produced by the major salivary glands of humans. Elucidation of the endocrine interactions between the human salivary glands and gonads is a long-term objective.

Changes in the morphology and functional status of the epithelial cells of the acini and ducts in the major salivary glands of rats in response to repeated amputation of incisors are transient [9]. This explains the gradual decrease in the severity of morphological changes in the spermatogenic epithelium and complete normalization of the ultrastructure of Sertoli and germ cells by the 6th week of the experiment.

CONCLUSION

We have shown that repeated amputation of incisors causes transient ultrastructural changes in Sertoli and spermatogenic cells of mature rats, similar to those observed after sialoadenectomy. The endocrine factors that make the greatest contribution to the regulation of spermatogenesis in rats are produced by cells of the granular convoluted tubules. Substances produced by the epithelial cells of the acini in the major salivary glands of rats probably have a less potent effect on spermatogenic epithelium.

REFERENCES

1. Perez P., Rowzee A.M., Zheng C., Adriaansen J., Baum B.J. Salivary epithelial cells: an unassuming target site for gene therapeutics. *Int. J. Biochem. Cell. Biol*. 2010; 42 (6): 773–777. DOI: 10.1016.
2. Sukmanskii O.I. Biologically Active Substances of the Salivary Glands. Kiev: Zdorov'e, 1991: 112.
3. Ivanova V.V., Milto I.V., Sukhodolo I.V., Usov V.Yu. Influence of Sialadenectomy on the Development of Spermatogenesis in Rats: Ultrastructural Characteristics of Spermatogenic Epithelium and Leydig Cells. *Bulletin of Experimental Biology and Medicine*. 2018; 166 (12): 773–777 (in Russ.). DOI: 10.1007/s10517-019-04444-w.
4. Afanas'ev V.V., Kalinchenko S.Yu., Vinokurova O.Yu., Ordashev Kh.A., Damadaev M.M. The state of salivary glands in patients with hypogonadism. *Russian Journal of Dentistry*. 2017; 21 (3): 137–140. DOI: 10.18821/1728-2802-2017-21-3-137-140.
5. Wells H., Zackin S.J., Goldhaber P., Munson P.L. Increase in weight of the submandibular salivary glands of rats following periodic amputation of the erupted portion of the incisor teeth. *Am. J. Physiol*. 1959; 196: 827–830. DOI: 10.1152/ajplegacy.1959.196.4.827.
6. Sun M.G., Williams J., Munoz-Pinedo C., Perkins G.A., Brown J.M., Ellisman M.H., Green D.R., Frey T.G. Correlated three-dimensional light and electron microscopy reveals transformation of mitochondria during apoptosis. *Nat. Cell Biol*. 2007; 9 (9): 1057–1065. DOI: 10.1038/ncb1630.
7. Rougeot C., Rosinski-Chupin I., Mathison R. Rodent submandibular gland peptide hormones and other biologically active peptides. *Peptides*. 2000; 21 (3): 443–455. DOI: 10.1016/s0196-9781(00)00158-3.
8. Mori M., Sumitomo S., Shrestha P., Tanaka S., Takai Y., Shikimori M. Multifunctional roles of growth factors or biologically active peptides in salivary glands and saliva. *Oral Medicine & Pathology*. 2008; 12 (4): 115–123. DOI: 10.3353/omp.12.115.
9. Ivanova V.V., Milto I.V., Sukhodolo I.V. Morphological and functional state of the epithelial cells in submandibular salivary glands after repeated amputations of the incisor teeth in adult rats. *Bulletin of Siberian Medicine.* 2018; 17 (1): 53–58 (in Russ.). DOI: 10.20538/1682-0363-2018-1-53-58.
10. Creasy D.M. Pathogenesis of male reproductive to-

xicity. *Toxicol. Pathol*. 2001; 29 (1): 64–76. DOI: 10.1080/019262301301418865.
11. Creasy D., Bube A., de Rijk E., Kandori H., Kuwahara M., Masson R., Nolte T., Reams R., Regan K., Rehm S., Rogerson P., Whitney K. Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol. Pathol*. 2012; 40 (6): 40–121. DOI: 10.1177/0192623312454337.
12. Franca L.R., Hess R.A., Dufour J.M., Hofmann M.C., Griswold M.D. The Sertoli cell: one hundred fifty years of beauty and plasticity. *Andrology*. 2016; 4 (2): 189–212. DOI: 10.1111/andr.12165.
13. Amaral A., Lourenco B., Marques M., Ramalho-Santos J. Mitochondria functionality and sperm quality. *Reproduction*. 2013; 146 (5): 163–174. DOI: 10.1530/REP-13-0178.
14. Lemus L., Goder V. Regulation of endoplasmic reticulum associated protein degradation (ERAD) by ubiquitin. *Cells*. 2014; 3 (3): 824–847. DOI: 10.3390/cells3030824.
15. Shubin A.V., Demidyuk I.V., Komissarov A.A., Rafieva L.M., Kostrov S.V. Cytoplasmic vacuolization in cell death and survival. *Oncotarget*. 2016; 7 (34): 55863–55898. DOI: 10.18632/oncotarget.10150.

Authors contribution

Ivanova V.V. – analysis and interpretation of data, justification of the manuscript. Tikhonov D.I., Serebrjakova O.N. – analysis and interpretation of data. Mil'to I.V. – conception and design of the study. Gereng E.A. – critical revision of the manuscript for important intellectual content. Pleshko R.I. – final approval of the manuscript for publication.

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