Morphophysiological features of spermatozoa of male white rats under experimental stress

I Yu Arestova, E G Sharonova and M Yu Kupriyanova

Federal State Budgetary Educational Institution of Higher Education «Chuvash I. Yakovlev State Pedagogical University», 38, K.Marx str. 428000, Cheboksary, Russia

E-mail: nessizz@rambler.ru

Abstract. The results of research on the influence of stressful factors on the morphological parameters of spermatozoa are presented. A series of experiments was carried out using sexually mature outbred white rats (90 days of life). The experimental group of animals was subjected to combined stress (three days - hunger, after the third day - emotional stress, in accordance with The Forced Swim Test). The sperm count per unit volume of the epididymal suspension was calculated, activity was assessed under a microscope, and morphology was studied. The negative effect of stress factors on the number, motility and morphology of spermatozoa has been identified.

1. Introduction

A significant number of clinical observations and experiments confirm the tendency to an increase in the number of spermatogenesis disorders of male reproductive development [1-3].

When assessing the quality of ejaculate, researchers rely on the recommendations of the World Health Organization [4]. However, among researchers there is no consensus on the assessment of sperm morphophysiology [5]. Data on the morphological and functional characteristics of sperm cells vary among different authors. This fact may indicate that sperm are very sensitive to various factors. It has been identified that spermatogenesis and sperm morphology can be affected by various factors: physical [6], chemical [7, 8], biological [9], social [10, 11].

At the same time, the effect of stress on spermatogenesis has not been sufficiently studied to date, studies of the role of stress mainly concern female reproductive function. Undoubtedly, the ethical component complicates the study of the effect of stress on spermatogenesis. In this case, it becomes difficult to isolate the stress component of the disorder of this process. However, there are experimental data confirming the relationship between stress and impaired spermatogenesis [12-14].

Based on the foregoing, the goal of the current research was to study the morphophysiology of spermatozoa of male white rats under experimental stress.

2. Materials and methods

An experiment was carried out using sexually mature males of outbred white rats weighing from 250 to 320 g.

Animals for observations are selected on the basis of analogues and divided into two groups of 10 animals each: the first - control; the second - experimental. Work with experimental animals was
carried out in accordance with the "Rules for conducting work using experimental animals" (decree No. 755 of 08.08.77 of the USSR Ministry of Health).

The duration of the experiment was three days, during which the animals of the second experimental group were kept in the absence of food, with free access to water, experiencing hunger stress. At the end of the third day, all the animals of the experimental group were additionally put under emotional stress, in accordance with The Forced Swim Test (the animals swam for 3 hours in a plastic aquarium filled with water at a temperature of 22-24°C). After three days, all animals were withdrawn from the experiment in accordance with the principles of good laboratory practice (GOST R 53434-2009).

The object of the study was the testes of the control group (comparison group, 10 rats) and experimental (10 rats subject to combined stress). To obtain mature sperm, the appendages of the testis were cut in a 5% glucose solution with a volume of 1 ml at a temperature of 37°C. Then, using a sterile rubber tube, the epididymis was released from sperm [15, 16].

The following parameters were determined: using the Goryaev chamber the number of spermatozoa per unit volume of the epididymal suspension was calculated; activity assessment was carried out under a microscope; morphology was studied. For the purpose of micromorphological analysis in stained preparations in duplicate, 200 sperm cells were counted and measured. Among the counted sperm cells, the percentage of abnormal was determined, the teratozoospermia index (ITZ) was calculated as the ratio of the total number of detected abnormalities to the number of abnormal spermatozoa. Sperm smears were studied using a MIKMED-6 light-optical microscope with video visualization. Image input and analysis was carried out using a computer with the Micro View morphometric analysis software (LOMO-Microsystems, St. Petersburg).

The reliability of the results was carried out taking into account a small sample of animals using the Mann-Whitney U test. The average values of the indicators are given in the form $M \pm s$ ($M$ - average, $s$ - standard error). Assessment of the statistical significance of differences between the means was carried out at a critical level of $p = 0.05$.

3. Results and discussion

From the data obtained, it follows that in the control group, the number of sperm per unit volume of the epididymal suspension is $17.19 \pm 0.52 \cdot 10^6$, which is 35.1% more than in the group put under stress ($p < 0.05$).

Microscopic examination of the epididymal suspension of males who underwent hunger and “emotional” stress showed a decrease in sperm motility. Thus, the number of motile sperm cells in males of the second group was $7.18 \pm 0.16 \cdot 10^6$, and in animals of the intact group it was $8.44 \pm 0.33 \cdot 10^6$ ($p < 0.05$).

At the same time, actively moving spermatozoa, of all motile, in the studied samples of the epididymal suspension of control male rats was $53.68 \pm 2.51\%$, and in the samples of the biological material of animals of the experimental group - $37.68 \pm 3.15\%$ ($p < 0.05$).

An indicator that affects fertility is sperm viability. To assess sperm viability, the number of deaths per 200 cells was counted. Dead spermatozoa were detected after staining the homogenate smears with eosin.

A greater number of dead spermatozoa in smears of the homogenate of animals of the second group were noted in comparison with control animals. So, in animals of the control group, the number of dead cells was $7.7 \pm 0.75\%$, while in males exposed to stress it was $14.75 \pm 1.48\%$ ($p < 0.05$).

At the time of the study, the content of viable gametes in the control and experimental animals was, respectively: $92.3 \pm 0.68$ and $85.3 \pm 1.34\%$ ($p < 0.05$).

The content of pathological gamete forms was also identified in both control animals and rats of the second group. Among the deviations were noted such as: a defect in the head, neck, middle part and tail.

It was found that if in the control group there were $15.40 \pm 0.57\%$, then in the second $32.42 \pm 1.54\%$ ($p < 0.05$).
It was found out that the most common morphological abnormalities are: the end of the flagellum in the form of a loop and the presence of a cytoplasmic drop on the flagellum. These deviations from all abnormal spermatozoa in the samples of control animals accounted for, respectively: 6.07 ± 0.52 and 6.33 ± 0.38%, and in the samples of rats of the second group, 8.48 ± 0.45 (p <0.05) and 6.76 ± 0.7% (p>0.05).

It was revealed that spermatozoa with an acrosome abnormality (bloated acrosome, absence of an acrosome) were detected in the amount of 45.78 ± 3.52% of all abnormal sperm in rats of the second group, and 24.9 ± 2.13% were found in samples of control animals (p<0.05).

Such a disorder as the combination of flagellum anomaly in the middle part with a drop on the flagellum was observed in animals of the control group in 0.99 ± 0.17% of cases of all abnormal cells, in the second group in 3.99 ± 0.38% of cases (p<0.05).

The remaining abnormalities (bloated head, thin head, large head, inclined head, the presence of a cytoplasmic drop at the base of the head, double tail, tail with creases, short tail and lack of tail) account for sperm in the control group from 0.9 to 10.2%, and in the experimental - from 0.5 to 6.9%.

It was revealed that the ITZ in rats of the control group is less (1.4) compared with that of rats from the second group (2.07).

Despite the fact that the data on parameters reflecting sperm quality are very contradictory [17-19], researchers note that male fertility directly depends on the frequency of sperm morphological abnormalities [20]. Moreover, the mechanisms of formation of morphological deviations of gametes also affect sperm motility [21]. This, in particular, is confirmed by the results of our experiment.

The data obtained during the observations indicate the development of a response from the male reproductive system in animals to simulated stress conditions. Such a reaction may include a decrease in the number of spermatozoa in the homogenate of the appendage in experimental rats compared with control animals; a decrease in sperm motility and viability. This, as can be expected, will lead to a decrease in the fertilizing ability. In addition, the revealed morphological abnormalities in the form of a cytoplasmic drop on the flagellum and the twisted tail are anomalies that are included in the group of abnormalities most common in infertile couples [22].

In the presence of pathological forms of gametes in control animals, in rats of the second group there were more of them.

Similar changes, when exposed to various chronic stress factors, were found in previous studies [23].

4. Conclusion
Thus, it was found that in males exposed to stressful factors, the number of sperm cells in a unit volume of epididymal suspension is significantly larger compared to intact males. An increase in the number of certain abnormalities in sperm morphology in male rats indicates the effect of the studied simulated stress conditions.

It was revealed that a higher ITZ in rats exposed to stress factors is accompanied by a decrease in the number of spermatozoa in the appendage homogenate, a decrease in sperm motility and viability, and an increase in the number of abnormal forms.

Undoubtedly, the degree of change in the parameters of ejaculate in experimental animals under conditions of simulated stress depends on the strength and duration of stress exposure. At the same time, it has been shown that even a time-insignificant, but combined effect of stress factors leads to significant changes in the spermogram, and the quantitative and qualitative parameters of the seminal fluid can serve as a convincing criterion for the maladaptation processes occurring in the body under the influence of stress.

References
[1] Mirzakulov D S and Mirsakulov Sh S 2015 Vestnik of Osh State University 1 73-6
[2] Pashkova E Yu and Kalinchenko S Yu 2013 Effective pharmacotherapy 1 26-31
[3] Shevyrin A A 2018 Russian medical journal. Medical Review 2 (12) 30-5
4

[4] Artifeksov S B, Borodachieva I V and Sergeev M Yu 2017 Reproduction problems 23 (1) 80-3
[5] De Felice F, Marchetti C, Marampon F, Casciali G, Muzii L and Tombolini V 2018 Andrology doi:10.1111/andr.12562
[6] Mirkazulov D S 2014 Features of the influence of organochlorine compounds on the fertile function of men living in the Osh region (Bishkek)
[7] Tomas Jambor, Hana Greifova, Jana Bistakova and Norbert Lukac 2018 Endocrine Disruptors and Reproductive Health in Males, Endocrine Disruptors, Ahmed R. G., IntechOpen, DOI: 10.5772/intechopen.78538. Available from: https://www.intechopen.com/books/endocrine-disruptors/endocrine-disruptors-and-reproductive-health-in-males
[8] Nitkin D M, Rakevich M V, Koleda A G, Baturevich L V and Yuraga T M 2018 Laboratory Diagnostics Eastern Europe 7 (4) 517-26
[9] Osadchuk L V, Popova A V, Kleshechev M A and Osadchuk A V 2017 Sechenov Russian Physiological Journal 103 (8) 940-51
[10] Swan S H, Charlene Brazil, Erma Z Drobnis, Fan Liu, Robin L Kruse, Maureen Hatch, J Bruce Redmon, Christina Wang, James W Overstreet 2003 Environmental Health Perspectives 111 (4)
[11] Bryukhin G V, Sizonenko M L, Kustavinova E V 2014 Reproduction problems 5 22-5
[12] Denisova T G, Denisov M S, Lezhenina S V, Bushueva E V, Lyalina T S and Fedorov A A 2018 Acta Medica Eurasica 1 15-21
[13] Oganesyan M A, Skuratovskaya L N and Drozdov G A 2004 Pathophysiology and modern medicine 37
[14] Laskov D S, Bryukhin G V, Sizonenko M L and Alymov E A 2014 Reproduction problems 2 18-22
[15] Luckiy D L and Nikolaev A A 1999 Morphological study of ejaculate (AGMA: Astrakhan) 46
[16] Popova A V, Kleshcheyov M A, Osadchuk A V, Gutorova N V and Osadchuk L V Vestnik of Novosibirsk State University. Series: Biology, Clinical Medicine 9 (3) 47-54
[17] Arestova Inessa Y, Vladislav V and Alekseev 2014 Biology and Medicine 6 (1)
[18] Meeker J D, Singh N P, Hauser R 2008 J. Androl 29 (4) 140-46
[19] Khan M S, Ali I, Khattak A M, Ullah A, Khan M A and Javed A 2006 Gomal J. Medical Science 4 (1) 10-4
[20] Proshin C N, Stepanov G V, Novikova E N, Bairamov A A, Bychkov E R, Shabanov P D and Komyakov B K 2010 Andrology and Genital Surgery 11 (3) 71-5
[21] Jouannet P, Ducot B, Feneux D and Spira A 1988 Int. J. Androl 11 (5) 379-94
[22] Potemina T E, Kuznetsova S V and Lyalyaev V A 2009 Modern technological medicine 2 23-6