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# Morphometric Parameters of The Testis of 6-Month-Old Rats in The Norm and Under Radiation Exposure

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***Abstract***

*Assessment of the morphofunctional status of the testicles in chronic radiation sickness after prolonged exposure to ionizing radiation, comparison of the morphometric and morphological characteristics of these organs with different intervals and, thus, the development of an optimal algorithm for prevention, diagnosis and treatment are among the priority tasks facing scientists.* ***The aim of the study*** *to reveal the age-related morphofunctional features of the testes in radiation sickness.* ***Methods.*** *An experimental study was carried out on material taken from the testes of white non-linear rats. Animals were divided into 2 groups (n=64): Group I - (intact) control (n=33); II - group - rats that received radiation for 20 days from 71 days of age at a dose of 0.2 Gy (total dose was 4.0 Gy) (n=31).* ***Results.*** *Morphometry of the testicles showed that their mass, length and thickness in postnatal ontogenesis change unevenly. Comparison of the rate of weight gain and body length with the weight and volume of the testicles shows that with an increase in their volume, body weight increases more than length.*

**Keywords:** *Morphology, Testis, Radiation, Seminiferous Tubules.*

## 1. Introduction

According to the World Health Organization, 9% of families worldwide are struggling with fertility problems, and it is the male factor that is the cause of 50% of the problems [1]. Male infertility has many causes, ranging from genetic mutations to lifestyle choices, the influence of various chemical and physical factors. Among these, ionizing radiation has a special role as it is classified as a Group 1 carcinogen by the International Agency for Research on Cancer and is listed by the International Labor Organization as an occupational carcinogen. Despite this, the number of radiation facilities and the number of their employees are also increasing by about 10 and 4% per year, respectively [2]. This once again emphasizes that reproductive health and related aspects are not only a medical but also a social problem.

All over the world, special attention is paid to research aimed at improving the early detection, treatment and prevention of diseases of the male reproductive system, including the testes, caused by various physical factors. In this regard, determine the amount of ionizing radiation, which leads to pathological changes in the male body; development of a system for monitoring the health of workers working with radiation sources; determine the level of risk of infertility and the occurrence and development of tumors in these individuals; study of the mechanisms of action of drugs that reduce the effects of radiation remains a priority of scientific research. Assessment of the morphofunctional status of the testicles in chronic radiation sickness after prolonged exposure to ionizing radiation, comparison of the morphometric and morphological characteristics of these organs with different intervals and, thus, the development of an optimal algorithm for prevention, diagnosis and treatment are among the priority tasks facing scientists [3,11].

**The purpose of the study:** to reveal the age-related morphofunctional features of the testes in radiation sickness.

## 2. Materials And Methods

An experimental study was carried out on material taken from the testes of white non-linear rats, which were kept in a vivarium under a 12-hour light regimen, with a standard diet and free access to water. At

the beginning of the experiment, all sexually mature rats were quarantined for a week, and after the exclusion of somatic or infectious diseases, they were transferred to the usual mode of the vivarium. Animals were divided into 2 groups (n=64): Group I - (intact) control (n=33); II - group - rats that received radiation for 20 days from 71 days of age at a dose of 0.2 Gy (total dose was 4.0 Gy) (n=31)

In the experimental groups, rats were irradiated to simulate chronic radiation sickness. Manipulation began at 71 days of age in rats and continued for 20 days at a fractional daily dose of 0.2 Gy (total dose was 4.0 Gy) up to 90 days of age using the AGAT R1 DTGT apparatus (Baltiets plant, Narva, Estonia, manufactured in 1991, in operation since 1994, recharged in 2007, with a capacity of 25.006 cGy / min.).

Animals were slaughtered at the appropriate time in the morning, on an empty stomach by means of instantaneous decapitation under ether anesthesia. After opening the abdominal cavity, the testes were removed and their mass, length, width, volume and tissue density were studied. The mass of each of the testes was measured on an electric scale, the length and width were measured with a millimeter tape.

The volume of the testicles according to the formula:

$V= 0.523 \times n \times c^2$,

where: n,c - respectively, the length and thickness of the testicle 0.523 - a constant coefficient.

The extracted testes were fixed in Bouin's solution. After passing through alcohols with an ascending concentration, they were embedded in hot paraffin, then sections were prepared from the testes with a standard thickness of 6–7 μm, which were oriented sagittally or frontally. Sections were stained with hematoxylin and eosin according to van Gieson. Finished histological preparations were examined under an NLCD-307B binocular microscope (Novel, China).

The diameter of rounded transverse sections of the convoluted seminiferous tubules was measured using an eyepiece micrometer DN-107T. The shortest distance between two points was measured, which were in the opposite diameter and lie between the basement membrane (inner part) and germ cells. To assess the average diameter of the convoluted seminiferous tubules, rounded transverse sections of the tubules were selected in different zones of randomly selected sections of the testis. Based on the data obtained, the cross-sectional area of the convoluted seminiferous tubules was calculated using the formula:

$S=P \times d^2 /4$,

where: S is the area of a separate section of the tubule, d is the diameter of a separate section of the tubule, P= 3.14.

To analyze the state of the spermatogenic epithelium in intact and experimental rats, the entire layer of spermatogenic epithelium was divided into 4 layers: 1 - spermatogonia, 2 - spermatocytes, 3 - spermatids, 4 - spermatozoa. Morphometric parameters of the convoluted seminiferous tubules and interstitial Leydig cells were determined on histological preparations, for which their number in the field of view was counted, and cell sizes were measured. The results obtained in the control group were compared with the data of the experimental groups.

The research materials were subjected to statistical processing using the methods of parametric and non-parametric analysis. Accumulation, correction, systematization of initial information and visualization of the obtained results were carried out in Microsoft Office Excel 2010 spreadsheets. Statistical analysis was carried out using the IBM SPSS Statistics v.23 program (developer - IBM Corporation).

**3. Results and Discussion**

In 180-day-old rats of the control group, body weight ranges from 210.69 to 225.03 g, on average - 218.3 + 1.021 g. The body length of rat pups was 16.19 - 19.3 cm, on average - 17.6 +0.280 cm.

The weight of the testes ranges from 0.99 to 1.35 g, on average 1.20 + 0.023 g. The length of the testes varies from 1.99 to 2.25 cm, on average 2.18 + 0.020 cm. 1.33 to 1.40 cm, on average it was 1.36 + 0.006 cm. The volume of the testes is on average - 2.57 + 0.015 $cm^3$.

In rats of 180 days of age under radiation, body weight ranges from 165.02 to 191.74 g, on average 179.1 + 2.473 g. Body length 14.01-16.43 cm (average - 15.0 + 0.221 cm). The mass of the testes is in the range from 0.89 to 1.00 g, on average - 0.95 + 0.009 g. The length of the testes ranges from 1.67 to 1.85 cm, on average - 1.77 + 0.016 cm. Thickness or the transverse size of the testes varied from 1.05 to 1.16 cm, on average - 1.11 + 0.010 cm. The average volume of the testes separately is 1.43 + 0.031 $cm^3$.

In rats of 180 days of age in the control group, the testes are oval. Outside, they are covered with a capsule, the thickness of which varies from 21.62 to 33.07 μm, on average 27.3 ± 0.970 μm. The basis of the capsule is loose, fibrous connective tissue, mainly consisting of reticular and collagen fibers. Elastic fibers are somewhat less common. The bundles of elastic fibers in the capsule of the testis are thinner than collagen fibers, directed longitudinally. The surface of this connective tissue layer is covered with 2-3 layers of squamous epithelium - mesothelium.

Beneath the albuginea of the testis are intrathecal vessels, consisting of arterioles and venules. It should be emphasized that these vessels are directed transversely and are located opposite the adnexal margin of the testis. Their number is 10 - 14, with a size from 136.1x136.1 to 144.21x144.21 μm, on average - 140.3 ± 0.693 μm. The walls of arterioles have a thickness that ranges from 21.33 to 30.32 μm, averaging 26.2±0.685 μm.

The distance between these arterioles varies from 374 to 783 μm. The subcapsular arterioles are the largest vessels of the testes. The wall of arterioles consists of 3 shells: inner, middle and outer. The inner shell consists of a single layer of endothelial cells with an elongated dark nucleus. Outside of it, a clearly defined folded elastic membrane is revealed. The middle shell is formed by 1-2 rows of spirally arranged smooth muscle cells. The outer shell is formed from fibrous connective tissue structures. The number of venules is 2 times less than the accompanying subcapsular arterioles. The diameter of the venules ranges from 26.4x38.5 to 42.3x60.02 μm, on average 58.5±0.241 μm.

The testis is based on convoluted seminiferous tubules, the diameter of which ranges from 186.4 to 235.13 μm, averaging 202.4±3.983 μm.

The cross-sectional area of the convoluted seminiferous tubules varied from 28568.7 to 35259.4 $\mu m^2$, on average – 32158.1±582.09 $\mu m^2$. The wall of the convoluted seminiferous tubules is based on reticular fibers. Collagen and elastic fibers are very poorly developed. On the inner side of the wall of the convoluted seminiferous tubules, spermatogenic epithelium is located in 1-2 layers. The thickness of the spermatogenic epithelium ranges from 45.98 to 80.05 μm, on average 62.3±2.388 μm. The height of spermatogonia cells is from 8.49 to 11.71 microns, on average - 9.8±0.282 μm. Spermatocytes are large cells with a size of 12.54 to 15.09 μm, on average 14.2±0.202 μm. And the size of spermatids is 8.34 - 10.17 μm, on average 9.3 ± 0.131 μm. The lumen of the seminiferous tubules is not free, there is a light zone 51.3-80.8 μm in size, on average 64.5±2.57 μm, which is filled with intercellular trophic substance.

In the intertubular spaces there are from 28 to 45, on average - 39.6 ± 1.319 Leydig cells with a size of 8.59 to 13.93 μm, on average - 10.7 ± 0.405 μm. In addition to these cells, arterioles and venules are also located in the intertubular spaces, which accompany the convoluted seminiferous tubules, as well as capillaries. The wall of intertubular arterioles has an average thickness of 10.1±0.226 μm, and their lumen ranges from 31.51x31.51 to 36.91x36.91 μm, on average 34.3±0.444 μm. The wall of arterioles consists of 3 membranes. The inner shell is represented by one row of elongated endotheliocytes, outside of it is a single convoluted elastic membrane.

The middle shell is formed by a single layer of smooth muscle cells arranged in a spiral. The outer shell consists of fibrous connective tissue structures. Intertubular venules accompany intertubular arterioles. In the wall of venules, one row of endothelial cells with elongated nuclei located at some distance from each other is determined. The wall thickness of the venules is on average 5.8±0.130 μm, and the lumen is 31.2±0.480 μm, with diameter fluctuations from 27.38x28.43 to 32.3x34.22 μm. The wall of the intertubular capillaries is formed by a single layer of endothelial cells with a flattened nucleus and located at a close distance from each other than in the wall of the venule. The lumen of the capillaries is on average 6.1±0.146 μm, and the wall thickness is 4.6±0.177 μm.

Reticular fibers, surrounding the accompanying vessels in the intertubular spaces, form a finely looped network. The bundles of collagen fibers are much thicker than the reticular ones, and the bundles of elastic fibers are found mainly around the arterioles and form the elastic membrane of the arterioles.

In 180-day-old male rats of the experimental group, the testes are oval and covered with a membrane, the thickness of which varies from 16.4 to 29.42 μm, on average - 23.2 ± 1.324 μm. Its wall is represented by bundles of collagen and elastic fibers. The bundles of elastic fibers are thinner and oriented mainly longitudinally. Thin reticular fibers in places form a finely looped network. From the outer surface, the shell is covered with 2-3 layers of longitudinally directed flat mesothelial cells.

Under the membrane of the testis there are intrathecal vessels: arterioles, which have a size from 127.94x127.94 to 132.43x132.43 μm, on average - 130.6 ± 0.433 μm. Their wall thickness varies from 18.03 to 32.04 μm, on average - 25.1±1.459 μm. These arterioles run transversely from the anterior,

free edge of the testis to the surface where the epididymis is located. The distance between arterioles ranges from 360 to 775 μm, and the number is 9-12.

The diameter of the venules is from 23.64x33.85 to 36.41x53.71 μm, on average 50.3±0.865 μm, the wall thickness is 5.4±0.259 μm.

The convoluted seminiferous tubules form the basis of the testis. Diameter - 144.52 - 192.22 μm (on average - 172.5 ± 4.014 μm). The cross-sectional area of the tubules is 20198.4 - 27443.6 $\mu m^2$ (average - 23358.6 ± 782.48 $\mu m^2$).

The basis of the wall of the tubules is represented by a fibrous structure of connective tissue, consisting of reticular fibers and bundles of collagen and elastic fibers. The reticular fibers around the seminiferous tubules form a network that resembles a "honeycomb" in transverse histological sections. The thickness of the spermatogenic epithelium ranges from 43.45 to 68.99 μm, on average 53.1±2.283 μm.

The first, more intensely stained layer of cells is spermatogonia. Their average size is 8.5±0.327 μm. The second layer is made up of larger cells - spermatocytes.

The third layer of spermatids, the average size of which is 8.3±0.120 μm. The free lumen of the seminiferous tubule (light zone) has a diameter of 42.6-61.5 μm, on average 55.6±2.13 μm.

Accompanying arterioles, venules, and capillaries are located in the intertubular spaces. Intertubular arterioles have a size from 29.71x29.71 to 33.47x33.47 μm, on average - 31.5±0.322 μm, their wall thickness averages 9.9±0.193 μm. The inner membrane of the arterioles is formed by endothelial cells with elongated nuclei located on the basement membrane. Outside of the inner shell is an internal elastic membrane, which has a distinctly pronounced folding. The middle shell is formed by one row of spirally arranged, smooth muscle cells, the nuclei of which are spindle-shaped. The outer shell is formed by loose connective tissue. The wall of the intertubular venules is formed by a single layer of endotheliocytes. The wall thickness of the venules is on average 5.3±0.220 μm, and the lumen diameter is 27.3±0.721 μm. The lumen diameter of the capillaries averages 5.8±0.378 μm. Their wall thickness is 3.9±0.225 μm. Leydig cells - endocrinocytes of the testis - in the intertubular spaces are located in the form of a triangle. The size of Leydig cells varies from 7.43 to 10.61 μm, on average - 9.2 ± 0.290 μm, and their number in the field of view ranges from 25 to 39, on average - 31.8 ± 1.269.

Reticular and collagen fibers in the intertubular spaces form a network, in the loops of which Leydig cells are located.

## 4. Conclusion

Morphometry of the testicles showed that their mass, length and thickness in postnatal ontogenesis change unevenly. Comparison of the rate of weight gain and body length with the weight and volume of the testicles shows that with an increase in their volume, body weight increases more than length. The mass of the testicles increases 1.16 times faster relative to body weight.

Irradiation, negatively affecting the morphological structures of the testes, leads to a lag in all morphometric parameters, including the thickness of the albuginea, spermatogenic epithelium. The lag is more pronounced in white outbred rats of 180 days of age.

In chronic radiation sickness, puberty is delayed. This is manifested by violations of the stages of spermatogenesis, changes in the cells of the spermatogenic series and late release of the lumen of the convoluted seminiferous tubules.

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