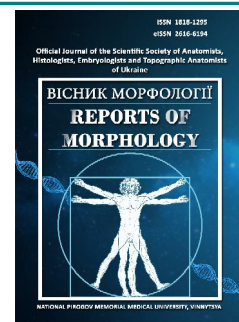




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Morphological structure of testicles under conditions of experimental gonadopathy and after the administration of cholecalciferol in comprehensive correction schemes

Marakhovskiy I. O., Korenieva Ye. M., Laryanovska Yu. B., Smolienko N. P., Belkina I. O., Velychko N. F., Chystiakova E. Ye., Misiura K. V., Bondarenko V. O.

SI "V. Danilevsky Institute for Endocrine Pathology Problems of NAMS of Ukraine", Kharkiv, Ukraine

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CORRESPONDING AUTHOR

e-mail: ihor1marahovskiy@gmail.com
Marakhovskiy I. O.

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The problem of male infertility is relevant and calls for a solution. The use of the D_3 vitamin in infertility treatment schemes has a potentially positive effect on reproductive health in male individuals. The present study aims to examine the effect of vitamin D_3 , administered alone or in combination with a preparation containing the extract of *Tribulus terrestris* on the changes in the histological picture of testicular morphology in rats with experimental gonadopathy. Male rats with modeled reproductive function pathology (Serotonin-induced gonadopathy) were divided into groups receiving correction using cholecalciferol alone or in combination with the reference drug Tribestan (Tr), which contains an extract of *Tribulus terrestris*. In addition to observational microscopy, morphometric evaluation of spermatogenesis was performed on sections of the testicles stained with hematoxylin and eosin. The statistical analysis was performed using the standard software package "Statistica 6.0" with the utilization of the Student's *t*-test and its nonparametric counterpart, the Kruskal-Wallis test for one-way analysis of variance, followed by the Mann-Whitney test. It was found that the seminiferous tubules of rats with Serotonin-induced gonadopathy are significantly reduced in size, and their tunica propria is thickened. Sertoli cells are often destructively altered, and the uniformity of their arrangement is disrupted. A decrease in the weight of the gonads, epididymis, prostate gland, and hypoandrogenization was observed as well as a decline in spermatogenesis indicators. An improvement in the morphological characteristics of the gonads was noted following the administration of vitamin D_3 in the presence of pathology. The seminiferous tubules had a normal histological structure. The germ cells were arranged in concentric rows according to their developmental stages, and the Sertoli cells appeared visually unchanged. The population of Leydig cells appeared visually more heterogeneous than in the control animals. However, occasionally seminiferous tubules with focal necrobiosis of germ cells and dystrophy of Sertoli cells, as well as a reduction in rows of germ cells, were observed. Overall, the quantitative indicators of spermatogenesis improved after the administration of vitamin D_3 compared to rats with experimental gonadopathy, although they did not reach the levels of the intact control. The administration of a combination of vitamin D_3 and Tribestan in the presence of gonadopathy resulted in a greater positive effect compared to their individual use. The microscopic condition of the testicular tissue in rats was fully recovered. The combined use of vitamin D_3 with Tribestan normalized the weight of the gonads and their appendages, significantly reduced the manifestations in the histological sections of degeneration and damage in the reproductive cells during the period of growth and differentiation, improved the relative level of androgen status in the organism and had a positive effect on spermatogenesis in the gonads. Thus, we have established that the combined use of Cholecalciferol and Tribestan for correcting experimental gonadopathy was more effective than either of the mentioned components alone.

Keywords: histological structure of the testis, gonadopathy, vitamin D, cholecalciferol, spermatogenesis.

Introduction

Over 48.5 millions of couples worldwide suffer from infertility due to reproductive dysfunction [25]. After 12 months or more of regular unprotected sexual intercourse without successful conception, the male factor alone or in combination with the female factor may be the cause of infertility in at least half of all cases. According to estimates, there are over 30 million infertile men worldwide [9]. Despite the alarming prevalence, the causes of male infertility remain in a significant percentage of cases undetermined. For instance, a prospective study of 1.737 infertile patients with abnormal semen parameters revealed the etiology of infertility in only 40 % of men [22]. The lack of a definitive diagnosis is particularly common among men with oligozoospermia. Approximately 75 % of oligozoospermia patients are diagnosed with idiopathic hypofertility [22], and this condition can be caused by gonadopathy of various origins. The impairment of the gonads causes a decrease in testicular function, accompanied by or resulting in changes in the morphostructure of the male reproductive glands, it causes hypofertility and results in a deficiency in the secretion of androgens, inhibin B, anti-Müllerian hormone, and spermatogenic insufficiency [9, 21].

Various correction methods are typically used in the treatment of male infertility, including hormonal and herbal remedies, cytomedines, essential amino acids, vitamins, etc [13]. However, despite significant advancements in pharmacotherapy for disorders of the reproductive glands, the issue of male infertility remains relevant and requires a solution, considering, in particular, the fact that its treatment improves the quality of life, holds high social significance, and reduces healthcare costs [17]. This prompts further research and exploration of new approaches, means, and strategies for the management of hypofertility. Recently, the use of vitamin D₃ for this purpose has been attracting the attention of researchers. Despite the prevalent occurrence of vitamin D₃ deficiency, the question of its impact on the reproductive function of the male body, specifically the morphological structure of the gonads and the germ cell formation within them, remains up for debate. The results of clinical and experimental data show that the deficiency of this vitamin-hormone negatively affects the development of testes and the process of spermatogenesis [11], determining the qualitative and quantitative parameters of the ejaculate [8, 19]. There is evidence that the use of vitamin D₃ can affect sperm motility in men with asthenozoospermia and low serum levels of 25(OH)D₃ [18]. There is an increasing number of publications highlighting the importance of vitamin D in sperm maturation [1, 12].

Vitamin D₃ deficiency in animals can lead to impaired maturation of seminal ducts, reduced testicular mass, and decreased sperm concentration [7], resulting in compromised reproductive ability in males.

At the same time, effective correction of gonadal morphology and testosterone levels in rats with induced aging, which leads to decreased fertility, has been reported

[14]. The addition of cholecalciferol (vitamin D₃) to the correction of reproductive pathology using linagliptin leads to significant recovery of testicular histoarchitectonics, normalization of steroidogenesis and spermatogenesis following experimental damage to testicular tissue by cisplatin [10].

Based on the above, *the aim of our study* was to determine the impact of vitamin D₃ used alone or in combination with a preparation containing an extract of *Tribulus terrestris*, on the histological changes in the morphological structure of rat testes with experimental gonadopathy.

Materials and methods

The study involved 30 sexually mature seven-month-old rats weighing 250-350 g. The animals involved in the study were collected from the experimental vivarium SI "V.Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine". The work was carried out following the national "General ethical principles of animal experimentation" (Ukraine, 2001), which are aligned with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985) [23]. The Wistar rat population was kept under standard vivarium conditions with natural lighting, a diet recommended for this species of animal, and unlimited access to water [15].

To model the pathology of reproductive function due to gonad impairment, a Serotonin model accompanied by impaired spermatogenesis was used.

The sexually active males were randomized into groups for this purpose: S-model group (animals with an experimental model of Serotonin-induced gonadal damage, gonadopathy (EGP)); S+vehicle (rats that received the vehicle (kernel oil) alongside the modeling of EGP) (for data analysis, these two groups were combined into one S group as they did not display any differences); S+vit. D₃ (animals that received vitamin D₃ orally alongside the modeling of Serotonin-induced testicular damage); S + Tribestan (Tr) (rats that received Tr as a reference drug alongside EGP); S+vit. D₃+Tr (animals that received both drugs orally: cholecalciferol and Tr alongside the modeling of EGP). The intact males were considered the control group.

The testicular pathology was induced by subcutaneous administration of Serotonin hydrochloride (ShanDong Octagon Chemicals Limited, China), for 14 days at a dose of 3.3 mg/kg body weight [26].

The reference drug Tribestan (Tr) was administered orally at a dose of 68 mg/kg body weight three days before the start of the Serotonin hydrochloride (SH) course, during the administration of Serotonin (14 days), and for three days after the last injection of Serotonin once a day. The dosage of the drug for the research was calculated using the species conversion factor based on the human daily

dose. Cholecalciferol (D_3) was administered following the same protocol, at a volume of 0.5 ml with a dose of 4000 IU (*per os*). The solutions made from kernel oil were prepared using vitamin D_3 (powder, China, batch CHG20062009, which complies with the quality standard GB 9840-2017).

At the end of the treatment period, the animals were euthanized by rapid decapitation, and their organs (testes, epididymides, ventral part of the prostate gland) were examined during autopsy to assess their condition and determine their weight.

The testes were removed and fixed in 10 % formalin for further histological investigations. According to the method described by B. A. Vartopetov and O. M. Demchenko (1975), the type of crystallization of the prostatic fluid (PF) was determined from prostatic imprints, the assessment of the "fern leaf" phenomenon allows evaluating the level of androgenic saturation in the animals' bodies [5]. To study the morphological structure, the fixed testes were dehydrated in progressively stronger alcohols and embedded in paraffin. Sections with a thickness of 6-7 μ m were obtained from the paraffin blocks using a sliding microtome MS-1, which were subsequently mounted on glass slides and stained with hematoxylin and eosin. Morphometric evaluation of spermatogenesis was performed in addition to the microscopic examination of testicular sections [2, 3].

The specimens were examined under the Granum L 30(03) light microscope, and microscopic images were captured using the Granum DCM 310 digital video camera.

The photographs were processed on a Pentium 2.4 GHz computer using the ToupView software.

The statistical analysis was performed using the standard software package "Statistica 6.0" with the utilization of the Student's t-test and its nonparametric counterpart, the Kruskal-Wallis test for one-way analysis of variance, followed by the Mann-Whitney test [16].

Results

Table 1 presents data on the organ weights of males with testicular injury induced with SH before and after correction. This pathology in males (group S) caused a decrease in the absolute weight of the testes by 20 % ($p<0.05$), their appendages (epididymides) by 16 % ($p<0.05$), and the ventral part of the prostate gland by 28 % ($p<0.05$) compared to those in the animals of the control group. The oral administration of vitamin D_3 or Tr (groups S+vit. D_3 and S+Tr) as monotherapy did not significantly alter the state of the investigated organs compared to the data of the animals in group S, indicating the continuation of the pathological process in them.

The combined use of vitamin D_3 with the baseline treatment (group S+vit. D_3 +Tr) as a correction for the pathological condition induced by experimental gonadopathy (EGP) increased absolute testes weight by 21 % ($p<0.05$) compared to animals in group S and even its normalization (see Table 1).

Table 1. Indicators of the absolute weight of the reproductive organs and pituitaries of male rats in the experimental groups ($M\pm m$).

Group	Testes, mg	Epididymides, mg	The ventral part of the prostate gland, mg
Control, n=5	3229 $\pm$ 186	1139 $\pm$ 38	500.6 $\pm$ 38.2
S, n=12	2582 $\pm$ 205.1	953.5 $\pm$ 40.9 ¹	362.0 $\pm$ 24.8 ¹
S+vit. D_3 , n=8	2767 $\pm$ 223	988.4 $\pm$ 35.0 ¹	402.4 $\pm$ 28.5 ¹
S+Tr, n=9	2932 $\pm$ 141	1041 $\pm$ 42	400.1 $\pm$ 27.8 ¹
S+vit. D_3 +Tr, n=12	3123 $\pm$ 1022	1033 $\pm$ 46	397.5 $\pm$ 40.5 ¹

Notes: ¹ - Statistically significant differences compared to the control group, $p<0.05$; ² - statistically significant differences compared to the S group, $p<0.05$; ³ - statistically significant differences compared to the S+vit. D_3 group, $p<0.05$; ⁴ - statistically significant differences compared to the S+Tr group, $p<0.05$.

It can be assumed, therefore, that the combined use of vitamin D_3 and Tr led to a reduction in the intensity of the pathological process in the testes, accompanied by the normalization of the weight of the gonads and their appendages, but did not affect the weight of the ventral part of the prostate gland. The effect of the mentioned composition on the weight of the testes, their appendages, and the prostate gland was comparable to that of the reference medication in rats.

Subsequently, the histological structure of the gonads in male rats, whose reproductive function was impaired by SH, as well as those receiving corrective therapy, was studied.

The testes of intact rats served as a general control in our studies on the effects of vitamin D_3 (Fig. 1) [4], administered both independently and in various correction schemes for experimental reproductive disorders, their morphological structure and morphometric indicators corresponded to normal values (Table 2).

As demonstrated by light microscopy on sections of intact rat testes, convoluted seminiferous tubules (T) were observed, cut in a transverse or oblique direction, and had an oval or round shape. The diameter of the T was normal, and the tunica propria of the T, as well as the proteinaceous and vascular membranes, were within normal limits. The wall of the seminiferous T consisted of germ cells. In the basal compartment, spermatogonia of the seminiferous epithelium were located. Among them, there are distinguishable cells with chromatin in the nucleus of condensed type, referred to as type B, and uncondensed type, known as type A. Type A spermatogonia present as both so-called "pale" (renewing) cells and "dark" (reserve) cells. Occasionally, mitosis can be observed in spermatogonia. In the intermediate compartment of the seminiferous tubule wall, spermatocytes are located. The majority of first-order spermatocytes were in the pachytene stage. In some tubules, metaphases of the first and (less frequently) second meiotic divisions, as well as anaphase of these divisions, were well evident. In the adluminal

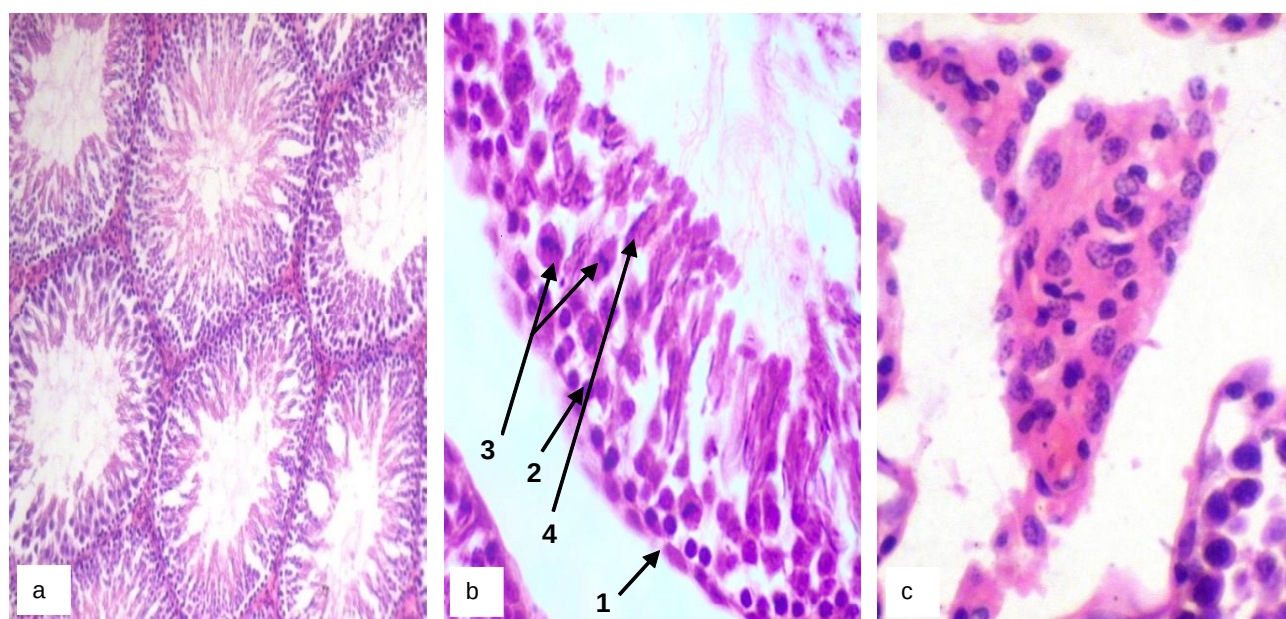


Fig. 1. The testes of intact rats: a - general view of seminiferous tubules (x100); b - in the epitheliospermatogenic layer of the tubule, Sertoli cells (1), spermatogonia (2), spermatocytes in metaphase I and II (3), and spermatids (4) can be observed (x250); c - normochromic Leydig cells in the intertubular space (x400). Hematoxylin-eosin [4].

Table 2. The impact of vitamin D3 on quantitative indicators of spermatogenesis in Serotonin-induced testicular pathology in rats (M±m).

Group	Indicators			
	Number of normal spermatogonia in the seminiferous tubule	Number of tubules in the 12th stage of meiosis, %	Number of tubules with exfoliated epithelium, %	Spermatogenesis index, scores
Control, n=4	61.08±0.48	4.000±0.413	0.000±0.000	3.331±0.002
S, n=8	23.46±4.26 ¹	0.748±0.252 ¹	5.253±0.371 ¹	1.652±0.344 ¹
S+vit. D ₃ , n=4	49.06±1.86 ^{1 2}	3.253±0.247 ²	2.002±0.410 ^{1 2}	3.022±0.131 ²
S+Tr, n=4	50.63±3.16 ^{1 2}	2.501±0.292 ^{1 2}	2.249±0.481 ^{1 2}	3.001±0.183 ²
S+vit. D ₃ +Tr, n=4	60.09±0.33 ^{2 3 4}	3.748±0.249 ^{2 4}	0.503±0.289 ^{2 3 4}	3.332±0.001 ²

Notes: ¹ - statistically significant differences compared to the data of the control group, $p<0.05$; ² - statistically significant differences compared to the data of the S group, $p<0.05$; ³ - statistically significant differences compared to the data of the S+vit. D₃ group, $p<0.05$; ⁴ - statistically significant differences compared to the data of the S+Tr group, $p<0.05$.

compartment of the seminiferous T, numerous spermatids and fully formed spermatozoa are visible. Germ cells at different stages of development are arranged in a strict order, forming concentric layers corresponding to the stages of the spermatogenic cycle. Associations of germ cells are clearly demarcated, and in different regions of the same T, only one specific combination of cells can be observed. In various tubules, not only spermatogenesis (the process of sequential transformation of germ cells: spermatogonia → spermatozoa) but also spermiogenesis can be clearly traced - the stages of cellular transformation from spermatids to spermatozoa. The strip of seminiferous epithelium contained at least 4-6 rows of cells. Numerous Sertoli cells (supporting cells) were located between the spermatogonia on the basal membrane. Their light pear-shaped nucleus with a nucleolus was clearly visible. The cytoplasmic extensions of these cells are masked by the germ cells at later stages of development. The interstitial connective tissue was very limited. Clusters of Leydig cells

(up to 7-20 cells), along with a few fibroblasts, were observed near the blood vessels within the interstitial spaces. The nuclei were mostly round to oval in shape, relatively large in volume, well-defined, normochromic, with noticeable evenly distributed chromatin granularity, and typically contained a single nucleolus. The cytoplasm of the cells was intensely eosinophilic, with weak peripheral vacuolization in some cells, and the cell boundaries were not clearly defined. The described state of Leydig cells indicates their normal functional activity (see Fig.1).

The morphometric characteristics of spermatogenesis in intact rats corresponded to the physiological norm for these animals (see Table 2).

After inducing gonadopathy by administering SH (group S) to 25 % of the rats, histological examination of the testes revealed a significant reduction in the size of seminiferous tubules, thickening, and swelling of their tunica propria. Sertoli cells were observed in the tubular wall (often with destructive changes, most of which appeared swollen, the

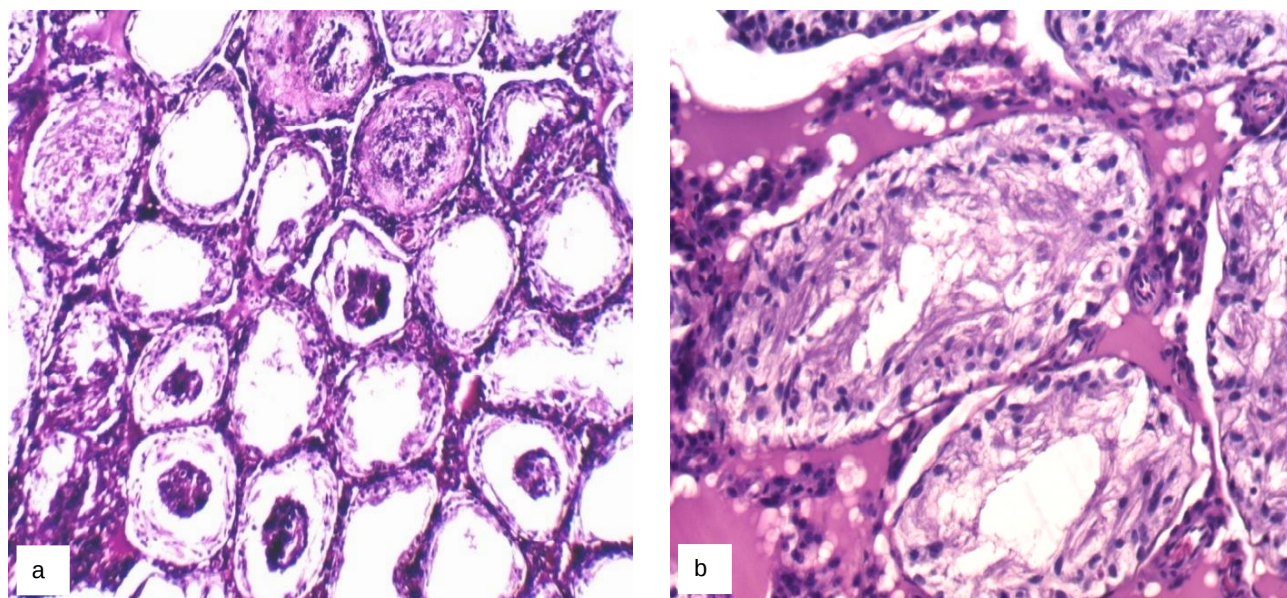


Fig. 2. Rat testis after administration of Serotonin hydrochloride: a - seminiferous tubules significantly reduced in size, the epitheliospermatogenic layer pathologically altered, some tubules filled with necrotic debris (x100); b - disordered arrangement of undifferentiated cells in the tubular wall, protein infiltration in the interstitial stroma (x250). Hematoxylin-eosin.

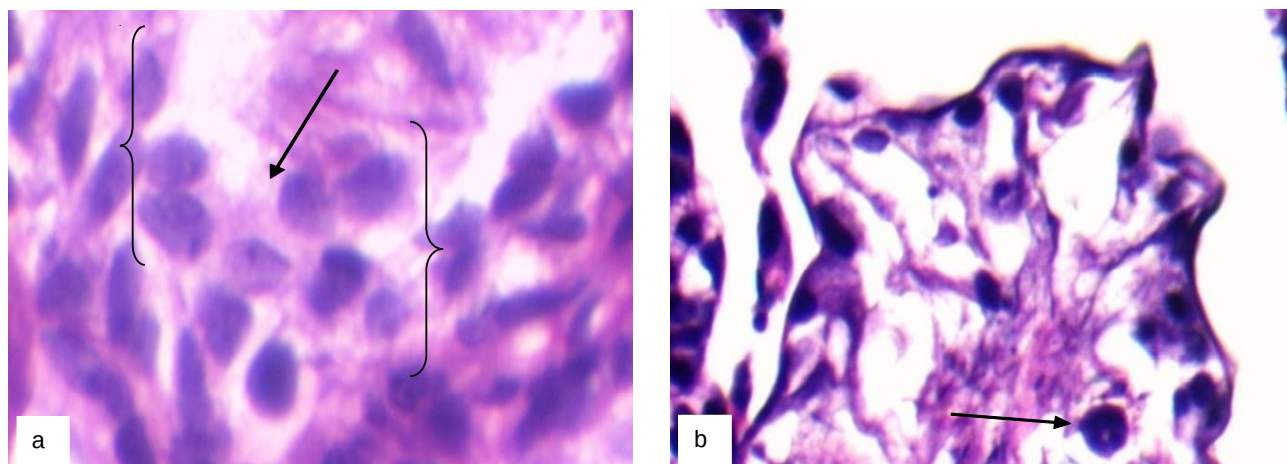


Fig. 3. Rat testis after administration of Serotonin hydrochloride: a - group arrangement of Sertoli cells; b - individual spermatid clumps in seminiferous tubules. Hematoxylin-eosin. x400.

nuclei sharply displaced or absent altogether, the regular arrangement of these cells was disrupted, with either individual cells or clusters present) and irregularly distributed undifferentiated cells of varying number. Some tubules were filled with necrotic debris. Accumulations of remnants of dystrophic germ cells were visible in the lumen of many tubules, while individual tubules exhibited "seminiferous syncytia" or cell clusters, characterized by giant multinuclear formations mainly containing spermatids. The intercellular stroma was extensively infiltrated with proteinaceous exudate (see Fig.2, Fig.3).

In the remaining 75 % of rats, a lesser degree of atrophy signs were found, and they exhibited significant variation. In the majority of those rats, the seminiferous tubules exhibited more or less preserved spermatogenic epithelium and complete destruction of the epitheliospermatogenic layer

was observed only in individual tubules or within the same tubule in a patchy pattern (the germ cells present in these foci appeared as shadow cell or were in a necrobiotic state). Frequently, in the seminiferous tubules, reduction of the germ cell rows, stratification of layers, and epithelial desquamation (disorganized displacement of structural elements into the lumen of the tubule) are observed, indicating a weakening of the intercellular connections between spermatocytes and spermatids with sustentocytes (see Fig. 3). In some tubules, there is a visually reduced presence of spermatogonia (including dark type A), very rare figures of mitosis in spermatocytes. There was a decreased presence of tubules with mature spermatozoa. There is a visually increased number of tubules in which a significant amount of residual bodies was observed in the lumen (remnants of cytoplasm shed by differentiating spermatids), indicating a weakened

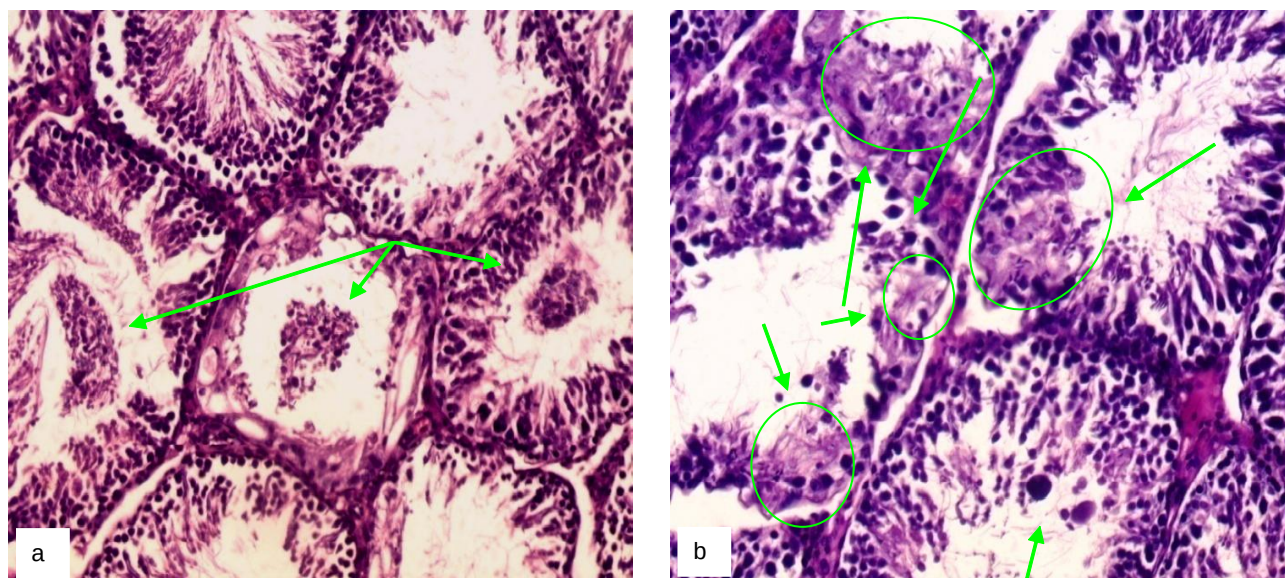


Fig. 4. Testes of rats after administration of Serotonin hydrochloride: against the background of tubules with relatively well-preserved epitheliospermatogenic layer, tubules with epithelial desquamation (a), nest-like destruction of the epithelium, and individual cell clusters were observed (b). Hematoxylin-eosin. x250.

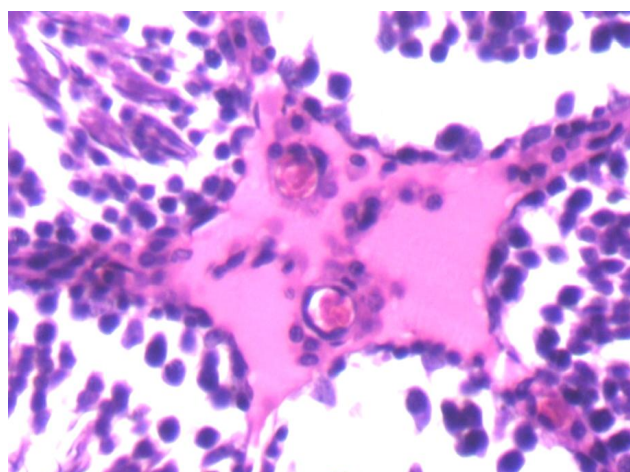


Fig. 5. Testes of rats after administration of Serotonin hydrochloride: decreased number of activated hormone-producing Leydig cells in the interstitial tissue, which is extensively infiltrated with protein. Hematoxylin-eosin. x400.

Table 3. The effect of vitamin D3 on the phenomenon of crystallization of the prostate gland secretion in Serotonin-induced testicular pathology in rats, scores ($M \pm m$).

Group	Crystallization of the prostate gland secretion
Control, n=8	3.612 ± 0.231
S, n=10	1.743 ± 0.262^1
S+vit. D ₃ , n=7	$2.448 \pm 0.030^{1,2}$
S+Tr, n=7	2.124 ± 0.061^1
S+vit. D ₃ +Tr, n=8	$2.669 \pm 0.024^{1,2,3,4}$

Notes: ¹ - statistically significant differences compared to the control group, $p < 0.05$; ² - statistically significant differences compared to the S group, $p < 0.05$; ³ - statistically significant differences compared to the S+vit. D₃ group, $p < 0.05$; ⁴ - statistically significant differences compared to the S+Tr group, $p < 0.05$.

function of their clearance by supporting cells. Syncytia are occasionally visible (Fig. 4, Fig. 5).

The quantitative assessment of spermatogenesis in Serotonin-exposed rats revealed changes in all its characteristic parameters: reduced number of normal spermatogonia in tubules, decreased number of tubules containing second-order spermatocytes in metaphase II of maturation (meiotic stage 12), increased frequency of tubules with sloughed epithelium. As a result, there is a significant decrease in the spermatogenesis index (see Table 2). It should be noted that the scoring of each cell layer in the tubular wall was conducted only if it contained at least 7 cells.

Androgen saturation of the organism, determined by the crystallization type of the prostate gland secretion, was significantly reduced in 25 % of rats. The phenomenon of "fern leaf" was not observed in the impression of the secretion, but isolated thick coarse cross-shaped crystals were detected against a structureless mass with small areas of "remnants" of distorted crystal branches. In the remaining 75 % of animals, the pattern of "fern leaf" was observed in the impressions, but the crystal branches were shortened, and less numerous, and the angle of divergence from the stems was significantly increased, indicating a considerable decrease in the level of androgen saturation in the organism. Overall, the assessment of crystallization of the prostate gland secretion was 1.76 scores for the group (see Table 3).

In animals with EGP after administration of the Serotonin hydrochloride and kernel oil, the microscopic picture of testicular tissue, spermatogenesis indicators, and level of androgenization based on the type of crystallization of the prostate gland secretion showed practically identical characteristics to the previously presented indicators,

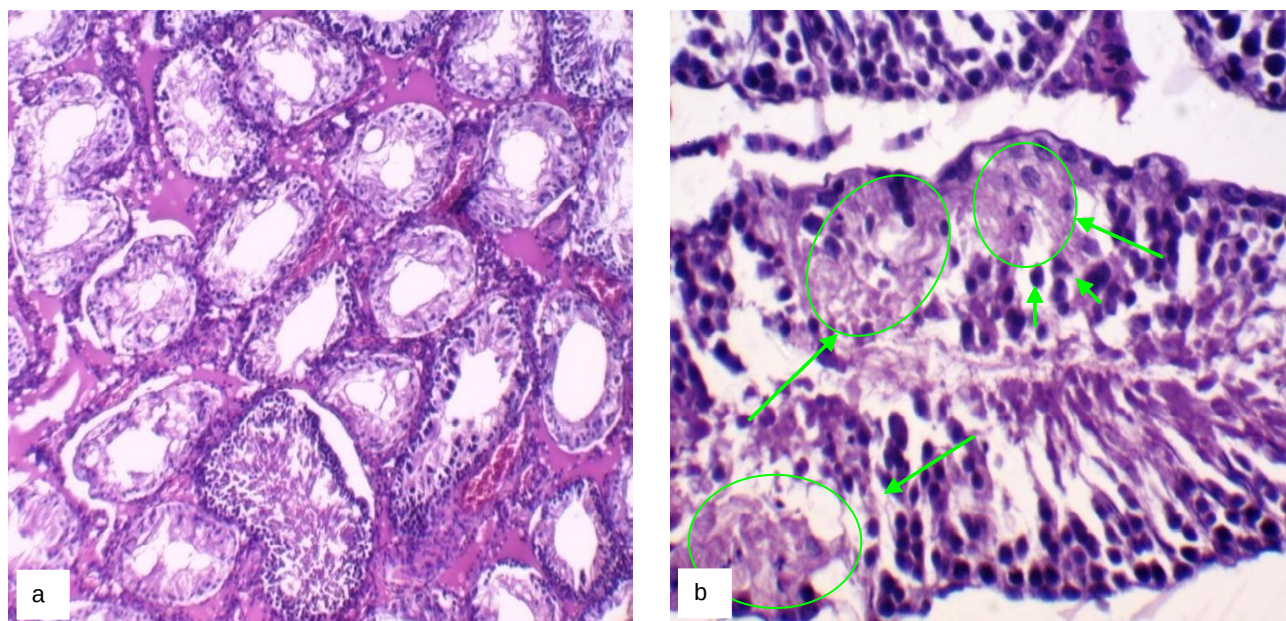


Fig. 6. Testes of rats after administration of Serotonin hydrochloride and kernel oil: a - decrease in the size of seminiferous tubules, atrophy of the spermatogenic epithelium, protein infiltration of the interstitial stroma (x100); b - nesting destruction of germ cells (x250). Hematoxylin-eosin.

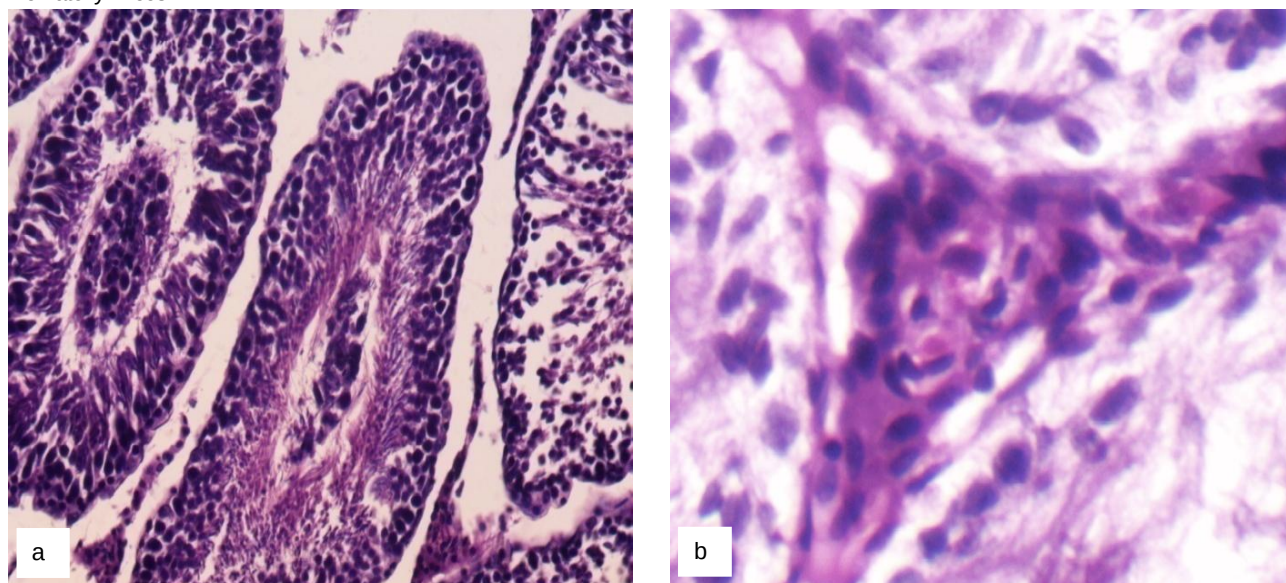


Fig. 7. Testes of rats after administration of Serotonin hydrochloride and kernel oil: a - desquamation of the epithelium into the lumen of seminiferous tubules (x200); b - monomorphic, less active Leydig cells in the interstitial tissue (x400). Hematoxylin-eosin.

including the distribution of animals according to the severity of pathology (see Fig. 6, Fig. 7, Table 3).

After the administration of D_3 in rats with the development of pathology, an improvement in the morphological picture of testicular tissue was observed. Seminiferous T were visually of normal size and had a normal histological structure. The epitheliospermatogenic layer contained germ cells arranged in concentric rows according to their developmental stages (including spermatozoa). The number of T with metaphase of the second meiotic division was increased (see Fig. 8). Sertoli

cells were visually unchanged. The population of Leydig cells appeared visually more heterogeneous than in the control animals. The majority of Leydig cells were activated, as evidenced by the presence of large round normochromic nuclei with distinct nucleoli. The cytoplasm of these cells had a larger volume (see Fig. 9). However, in all animals with fully restored spermatogenesis, there were areas of focal necrobiosis of germ cells, dystrophy of supporting cells specifically in the regions of destruction (see Fig. 10), reduction of germ cell rows, layer disorganization, epithelial desquamation, and the presence of few cell

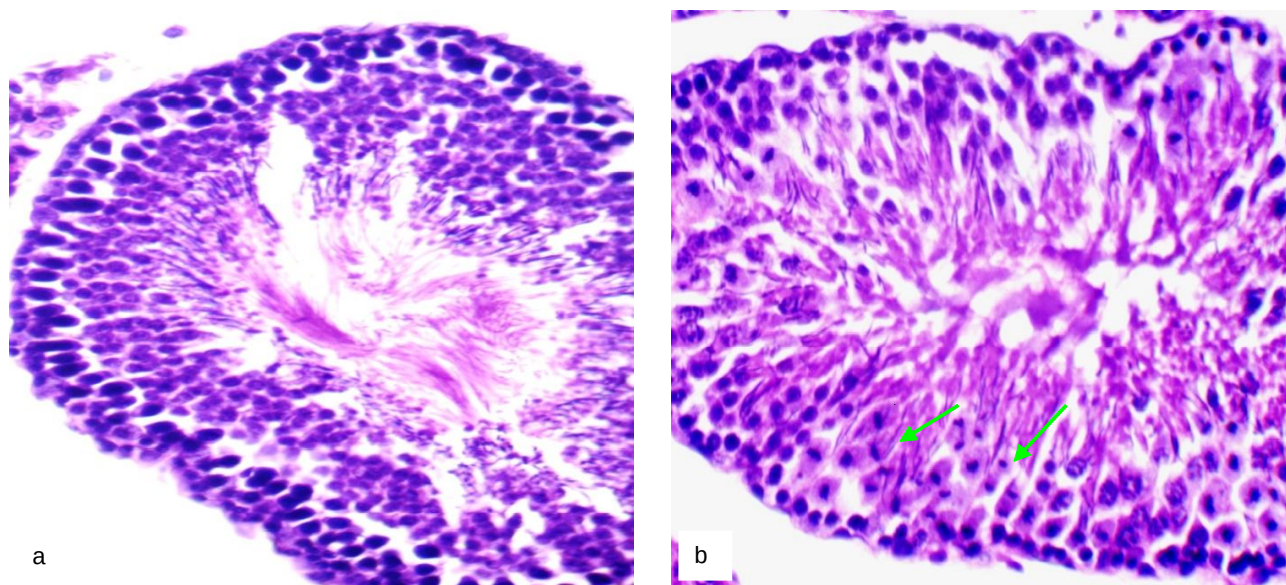


Fig. 8. The testes of rats after administration of Serotonin hydrochloride and vitamin D₃: a - a complete pool of germ cells in the seminiferous tubule (x200); b - numerous secondary spermatocytes in the metaphase of the second division (x250). Hematoxylin-eosin.

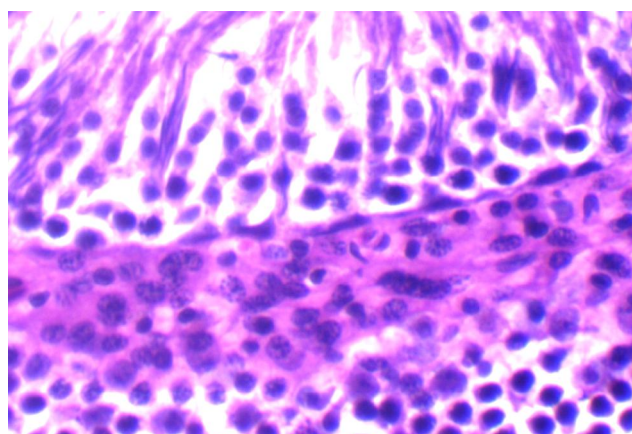


Fig. 9. The testes of rats after administration of Serotonin hydrochloride and vitamin D₃. The majority of Leydig cells are in an active functional state. Hematoxylin-eosin. x400.

clusters (see Fig.11). The severity of these impairments varied among different males within the group, but overall, they were more pronounced in animals from the EGP group, as confirmed morphometrically: the quantitative indicators of spermatogenesis improved after D₃ administration compared to rats with S-GP, although they did not reach the levels of intact control. For instance, the number of normal spermatogonia in the seminiferous tubules increased by 2.1 times. As a result, a significantly greater number of secondary spermatocytes were able to undergo division, and the number of tubules with the 12th stage of meiosis increased. The spermatogenesis index increased by 1.95 times (see Table 2).

The reference drug Tr, administered following a similar scheme, exerted a sufficiently pronounced positive effect on the state of the testicular tissue in rats with EGP. In 50 % of the animals, cross-sections of the seminiferous tubules

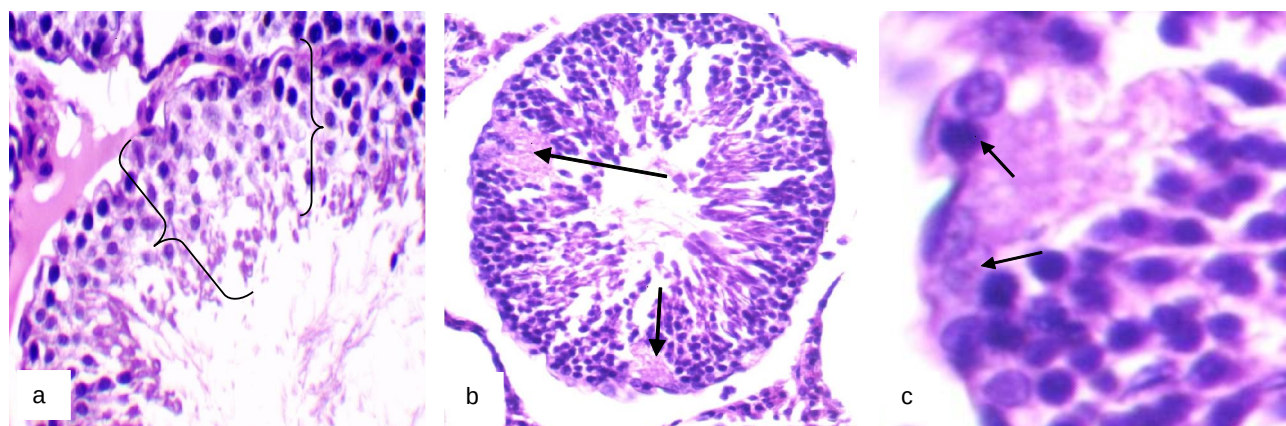


Fig. 10. The testes of rats after administration of Serotonin hydrochloride and vitamin D₃. Varying degrees of destruction of germ cells at various stages of development in the seminiferous tubules (a - b, x250, x200), dystrophy of Sertoli cells in the area of epithelial destruction (c, x400). Hematoxylin-eosin.

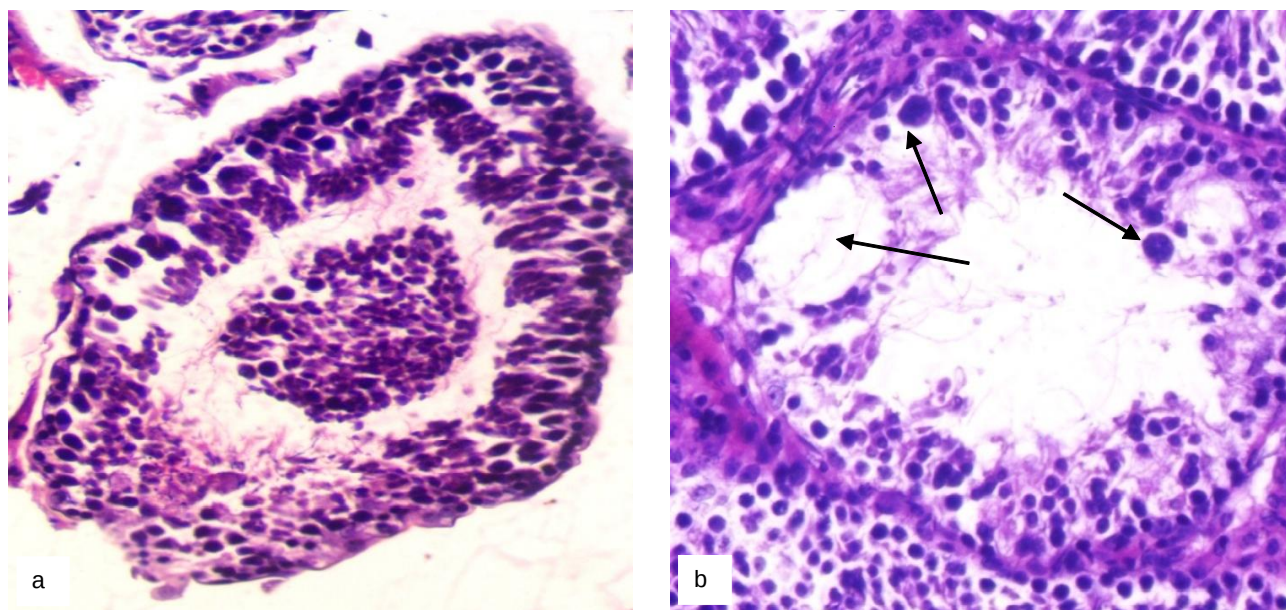


Fig. 11. The testes of rats after administration of Serotonin hydrochloride and vitamin D₃: a - desquamation of epithelium, focal layering of cell layers; b - focal reduction of germ cell rows, syncytia. Hematoxylin-eosin. x250.

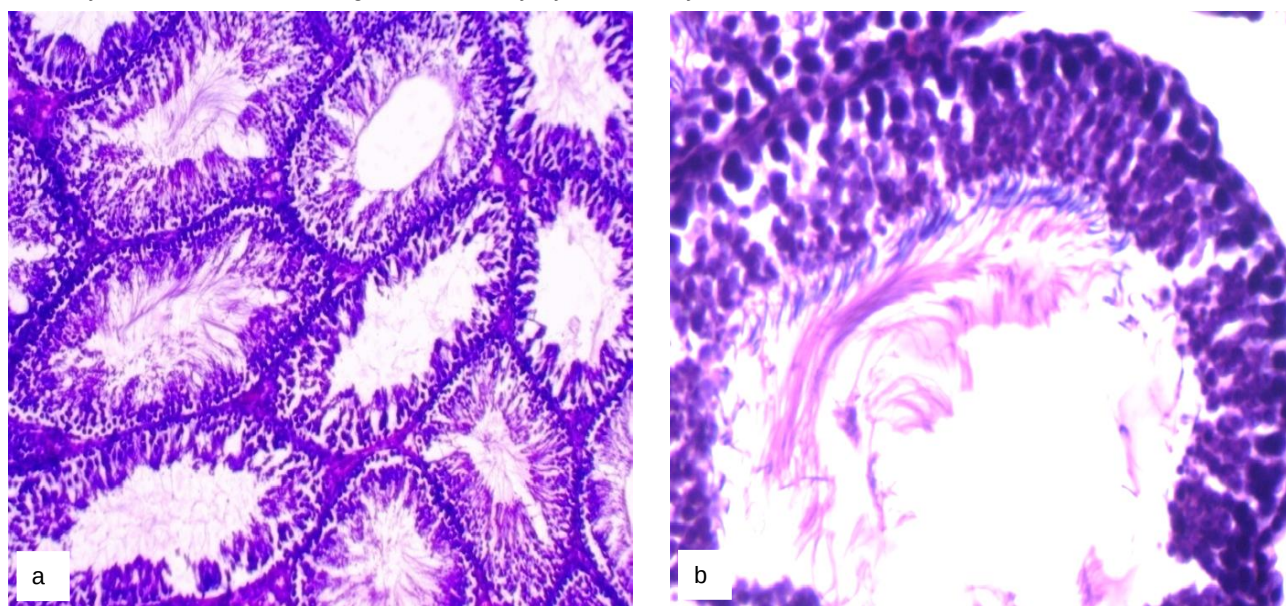


Fig. 12. Rat testis after Tribestan administration in combination with Serotonin hydrochloride: a - the normal state of seminiferous tubules (x200); b - the full spectrum of germ cells (x250). Hematoxylin-eosin.

revealed an unchanged strip of the spermatogenic epithelium, characterized by its width, organization, and completeness of the represented pool of germ cells.

In the rest of the rats, seminiferous tubules of varying number with areas of epithelial disorganization were found, reduction in rows of germ cells, and their stratification. The typical combination of different cell types was not disrupted (Fig. 12).

The areas varied in size, clearly showing a decrease in the number of spermatogonia. The nuclei of Sertoli cells acquired a rounded shape, with displaced nuclei (Fig. 13). The glandular cells varied in number, size, and nuclear

status, with an increased presence of inactive forms in rats with changes (Fig.14).

The quantitative indicators of spermatogenesis after administration of Tr overall confirmed the visual assessment of the testicular tissue condition: the number of normal spermatogonia was significantly increased compared to the pathological control, there was an increased meiotic activity in the seminiferous tubules, minimal epithelial desquamation was observed, and the spermatogenesis index was increased. However, all these indicators did not reach the level of intact control (see Table 2).

The administration of a combination of vitamin D₃ and

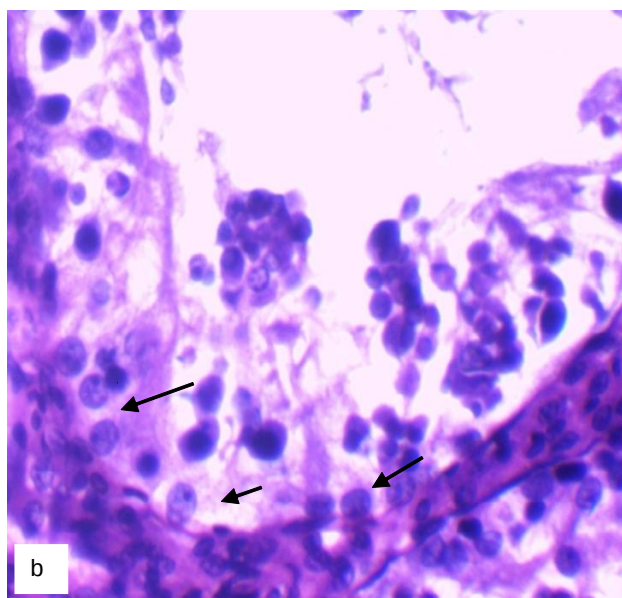
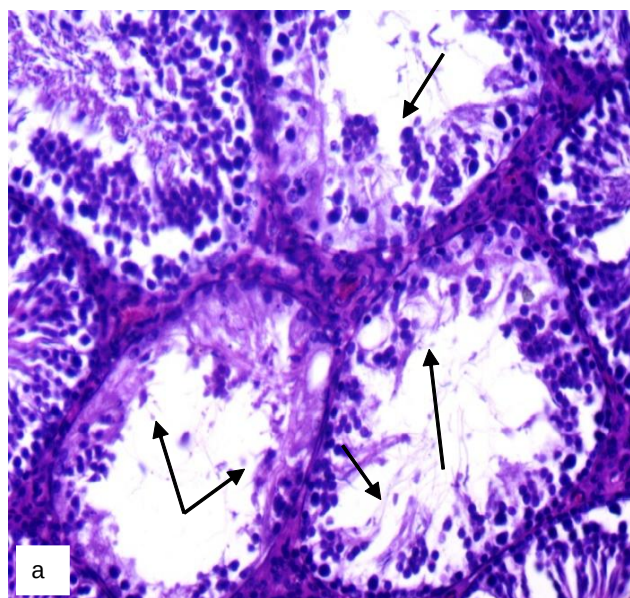


Fig. 13. Rat testis after Tribestan administration in combination with Serotonin hydrochloride: a - necrobiosis of germ cells, reduction of rows, the appearance of "windows" in the epitheliospermatogenic layer of the tubules (x250); b - dystrophic changes in Sertoli cells (x400). Hematoxylin-eosin.

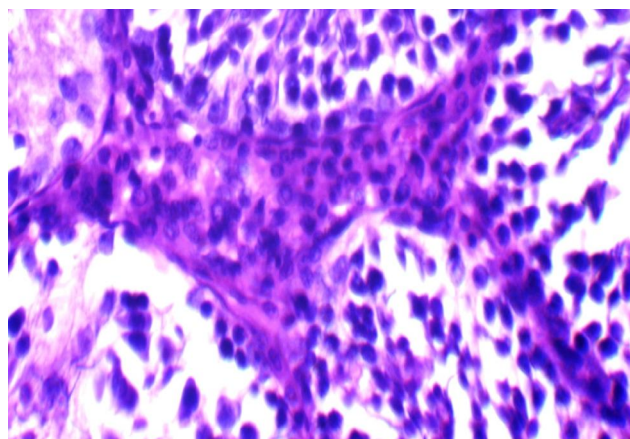


Fig. 14. Rat testis after Tribestan administration in combination with Serotonin hydrochloride. Increase in inactive forms of Leydig cells in the interstitial space. Hematoxylin-eosin. x250.

Tr on the background of EGP potentiates the positive effect of each component when used alone. In all 100 % of rats, the microscopic condition of the testicular tissue was completely restored. The size of the seminiferous tubules, the width of the epithelial spermatogenic layer, the presence of germ cells, their arrangement, and the typical combination of cell types corresponded to the norm. Seminiferous tubules with spermatogenic cells showing dystrophic changes or in a state of necrobiosis were not observed in all animals, and they were sporadic (see Fig. 15). There were no visually identifiable changes in Sertoli cells (see Fig. 16).

Leydig cells and quantitative characteristics of spermatogenesis have been restored nearly to the intact level in these rats (see Table 2).

As for the level of androgen saturation in their organisms, in all rats of this group, the "fern leaf" pattern has been practically fully restored. Only small areas with contracted, reduced branching, and increased angle of departure from the main stem have remained. The evaluation of the pattern was 2.67 points (see Table 3).

Discussion

Summarizing the obtained results, the following observations can be made: long-term administration of Serotonin hydrochloride in a dose of 3 mg/kg leads to the development of gonadopathy, accompanied by a decrease in the mass of testes, their appendages, and the ventral part of the prostate gland, and the occurrence of pathological changes in the testicular tissue of rats, characterized by destructive alterations of Sertoli cells (which are responsible for nourishing the germ cells [20]), varying degrees of destruction of seminiferous tubules, dystrophy and necrobiosis of germ cells, as a result, the spermatogenesis is suppressed, including a reduction in the number of stem cells (spermatogonia), delayed differentiation of germ cells (spermatids ® spermatozoa), and a decrease in the spermatogenic index. The obtained results are supported by the literature data on the histological structure of the testes and the submicroscopic architecture of Sertoli cells after the use of Serotonin hydrochloride [6, 26]. Under the influence of Serotonin hydrochloride, the number of activated hormone-producing Leydig cells decreases, as observed in our study and reported in the literature, which leads to a decrease in the androgen status of the animals' bodies, and is confirmed not only indirectly by the test of crystallization of the prostate gland secretion (see Table 3) but also by a decrease in the

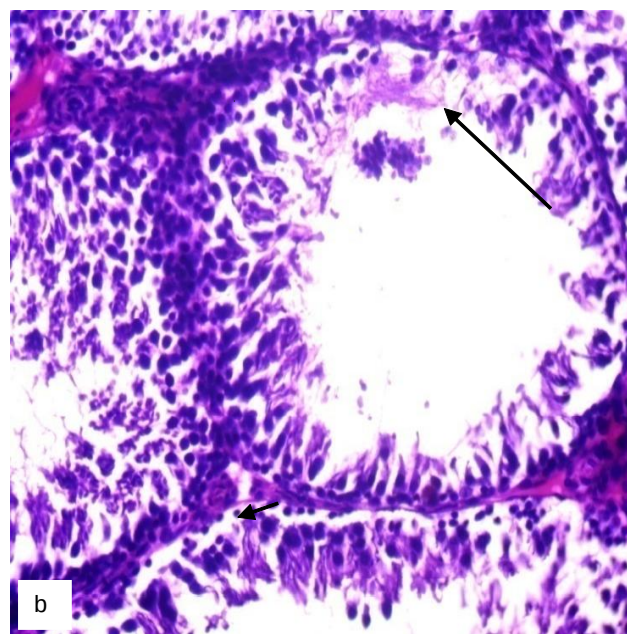
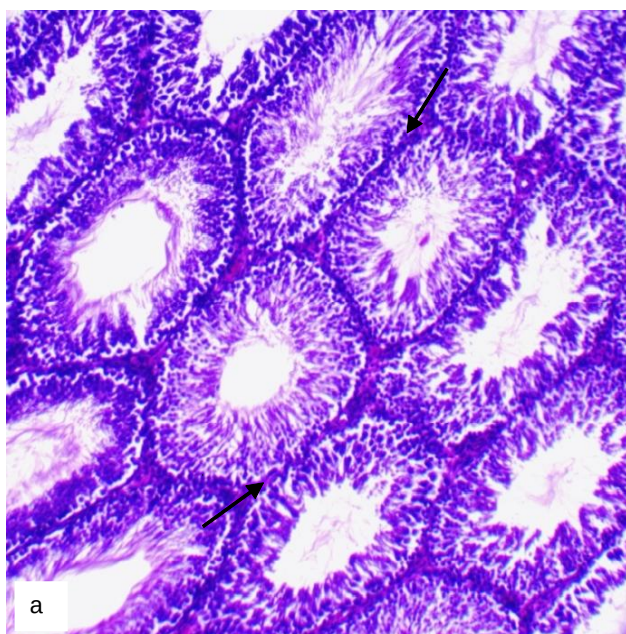


Fig. 15. Rat testis after administration of a combination of vitamin D₃ and Tribestan on the background of Serotonin hydrochloride: a - normal state of testicular tissue (x100); b - individual seminiferous tubule with focal destruction of germ cells (x200). Hematoxylin-eosin.

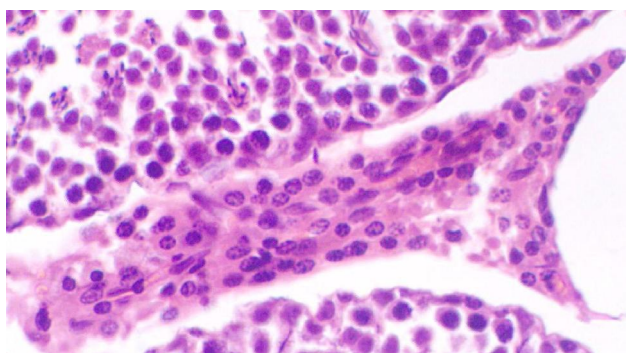


Fig. 16. Rat testis after administration of a combination of vitamin D₃ and Tribestan on the background of Serotonin hydrochloride. Prevalence of active forms of Leydig cells. Hematoxylin-eosin. x400.

level of male sex hormone associated with experimental gonadopathy [4].

The administration of vitamin D₃ does not affect the mass indicators of the reproductive organs. However, in the gonads of animals in this group, the number of normal spermatogonia increases, the percentage of seminiferous tubules with exfoliated epithelium decreases compared to the pathology but does not reach the level of intact animals, while the percentage of tubules in the 12th stage of meiosis and the spermatogenesis index normalize. After the administration of cholecalciferol in EGP, the condition of Sertoli cells improves, which patently contributes to the improvement of trophic support for germ cells, resulting in reduced dystrophic and destructive manifestations in germ cells during growth and differentiation, which in turn leads to increased reserve of spermiogenesis, an elevated spermatogenesis index, and noticeable activation of

hormone-producing Leydig cells, which is the most likely cause of an increase in the androgen status of the animals' bodies [6]. In terms of the positive effects on the morphological state of the testes, spermatogenesis in the gonads, and the androgen status of the organism, vitamin D₃ when administered separately, is practically equal to the reference drug Tr, and when used in combination with the investigated preparations, the effect is potentiated. Positive changes in the morphological structure of the rat testes, observed in the group receiving combined correction (S+D₃+Tr), contribute to the increase in the level of spermatogenesis [24] and improvement of the reproductive potential of animals with Serotonin-induced gonadopathy.

Conclusion

1. Modeling testicular hypofunction using Serotonin hydrochloride leads to the development of pathological changes in the testicular tissue of rats. Histological examination of the testes reveals a significant reduction in the size of seminiferous tubules, thickening and swelling of their tunica propria. Sertoli cells are frequently destructively altered, and the uniformity of their arrangement is disrupted. The interstitial stroma is abundantly infiltrated with proteinaceous exudate. A decrease in the weight of the testes, epididymides, and prostate gland is observed compared to intact rats, along with hypoandrogenization and a decrease in all indicators of the spermatogenesis process.

2. After the administration of D₃ in the presence of pathological changes, an improvement in the morphological picture of the testicular tissue was noted. The seminiferous tubules exhibited a normal histological

structure. The epitheliospermatogenic layer contained germ cells arranged in concentric rows according to their developmental stages, including spermatozoa. The number of tubules with metaphase of the second division of maturation was increased, and Sertoli cells appeared visually unchanged. The population of Leydig cells was visually more heterogeneous than in control animals. The majority of Leydig cells were activated, as indicated by the presence of large round normochromic nuclei with distinct nucleoli. However, in all animals, alongside tubules with fully restored spermatogenesis, there were also tubules showing focal necrobiosis of germ cells and Sertoli cell dystrophy, accompanied by a reduction in the germ cell rows. Overall, the quantitative indicators of spermatogenesis improved after the administration of D₃ compared to rats with experimental gonadopathy, although they did not reach the levels of the intact control. The weight of the reproductive organs did not differ from the measurements of animals with pathology.

3. The reference drug, when administered according to a similar scheme, had a pronounced positive effect on the state of the testicular tissue in rats with experimental gonadopathy. In 50 % of the animals, cross-sections of the

seminiferous tubules showed an unchanged strip of spermatogenic epithelium in terms of width, organization, and completeness of the represented pool of germ cells. The quantitative parameters of spermatogenesis in the tubules increased compared to rats with pathology, and the spermatogenic index in the gonads normalized. The typical combination of different cell types remained intact. Under conditions of Serotonin-induced gonadopathy in rats, the weight of the testes and epididymides normalized, and the weight of the prostate gland was equal to that in rats with pathology.

4. The combined use of vitamin D₃ together with Tribestan in experimental Serotonin-induced gonadopathy is accompanied by the normalization of gonad and appendage weights, it reduces histological manifestations of dystrophic and destructive changes in germ cells during growth and differentiation, improves the relative level of androgen status in the body, and has a positive effect on spermatogenesis in the gonads. Correction of Serotonin-induced gonadopathy with a combination of vitamin D₃ and Tribestan yields better results than using either of the mentioned components alone.

References

- [1] Aquila, S., Guido, C., Middea, E., Perrotta, I., Bruno, R., Pellegrino, M., & Ando, S. (2009). Human male gamete endocrinology: 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) regulates different aspects of human sperm biology and metabolism. *Reproductive Biology and Endocrinology*, 7, 1-13. doi: 10.1186/1477-7827-7-140
- [2] Bahriy, M. M., Dibrova, V. A., Popadynets, O. H., & Hryshchuk, M. I. (2016). *Методики морфологічних досліджень: Монографія [Methods of morphological research: Monograph]*. Вінниця: Нова книга - Vinnytsya: A new book.
- [3] Barylyak, I. R., Neumerzhyts'ka, L. V., Byshovets', T. F., & Danylenko, V. S. (2000). *Вивчення гонадотоксичної дії нових лікарських засобів та їх впливу на репродуктивну функцію тварин. Методичні рекомендації [Study of gonadotoxic effects of new drugs and their effects on reproductive function of animals. Guidelines]*. Київ: Авіцена - Kyiv: Avicenna.
- [4] Bielkina, I., Velychko, N., Marakhovskyi, I., Korenieva, Y., Smolienko, N., Chystiakova, E., ... & Bondarenko, V. (2023). The impact of cholecalciferol on the sex hormones level and serum biochemical indices in rat males with experimental gonadopathy. *Problems of Endocrine Pathology*, 80(1), 54-63. doi: 10.21856/j-PEP.2023.1.07
- [5] Brechka, N. M. (2019). Показники гормонального статусу самців щурів після застосування препаратів природного походження в умовах доброякісної гіперплазії передміхурової залози [Indicators of the hormonal status of male rats after the use of drugs of natural origin in conditions of benign prostatic hyperplasia]. *Український журнал медицини, біології та спорту - Ukrainian Journal of Medicine, Biology and Sports*, 4(5), 325-331. doi: 10.26693/jmbs04.05.325
- [6] Brechka, N. M., Nevzorov, V. P., Bondarenko, V. A., Malova, N. G., & Selyukova, N. Yu. (2015). Дослідження субмікроскопічної архітектури клітин Сертолі й Лейдіга після впливу гідрохлориду серотоніну та можливості корекції метаболічними засобами [Investigation of the submicroscopic architectonics of Sertoli and Leydig cells after exposure to Serotonin hydrochloride and the possibility of correction with metabotropic agents]. *Фізіологічний журнал - Physiological Journal*, 61(4), 93-99. doi: 10.15407/fz61.04.085
- [7] Chen, Y., & Zhi, X. (2020). Roles of vitamin D in reproductive systems and assisted reproductive technology. *Endocrinology*, 161(4). doi: 10.1210/endocr/bqaa023
- [8] Ciccone, I. M., Costa, E. M., Pariz, J. R., Teixeira, T. A., Drevet, J. R., Gharagozloo, P., ... & Hallak, J. (2021). Serum vitamin D content is associated with semen parameters and serum testosterone levels in men. *Asian Journal of Andrology*, 23(1), 52. doi: 10.4103/aja.aja_9_20
- [9] Crafa, A., Calogero, A. E., Cannarella, R., Mongioi, L. M., Condorelli, R. A., Greco, E. A., ... & La Vignera, S. (2021). The burden of hormonal disorders: a worldwide overview with a particular look in Italy. *Frontiers in Endocrinology*, 12, 694325. doi: 10.3389/fendo.2021.694325
- [10] Elrashidy, R. A., Zakaria, E. M., Elmaghraby, A. M., Abd El Aziz, R. E., Abdelgalil, R. M., Megahed, R. M., ... & Ibrahim, S. E. (2022). Linagliptin and Vitamin D₃ Synergistically Rescue Testicular Steroidogenesis and Spermatogenesis in Cisplatin-Exposed Rats: The Crosstalk of Endoplasmic Reticulum Stress with NF- κ B/iNOS Activation. *Molecules*, 27(21), 7299. doi: 10.3390/molecules27217299
- [11] Fu, L., Chen, Y. H., Xu, S., Ji, Y. L., Zhang, C., Wang, H., ... & Xu, D. X. (2017). Vitamin D deficiency impairs testicular development and spermatogenesis in mice. *Reproductive Toxicology*, 73, 241-249. doi: 10.1016/j.reprotox.2017.06.047
- [12] Hirai, T., Tsujimura, A., Ueda, T., Fujita, K., Matsuoka, Y., Takao, T., ... & Okuyama, A. (2009). Effect of 1,25-dihydroxyvitamin D on testicular morphology and gene expression in experimental cryptorchid mouse: testis specific cDNA microarray analysis and potential implication in male infertility. *The Journal of urology*, 181(3), 1487-1492. doi: 10.1016/

- j.juro.2008.11.007
- [13] Horpynchenko, I. I., Hurzhenko, Yu. M., & Imshynets'ka, L. P. (2019). Надання медичної допомоги хворим з чоловічим фактором безпліддя подружньої пари [Providing medical care to patients with a male infertility factor in a married couple]. *Здоров'я чоловіки - Men's Health*, (2), 23-35. doi: 10.30841/2307-5090.2.2019.179446
- [14] Jeremy, M., Gurusubramanian, G., & Roy, V. K. (2019). Vitamin D3 mediated regulation of steroidogenesis mitigates testicular activity in an aged rat model. *The Journal of Steroid Biochemistry and Molecular Biology*, 190, 64-75. doi: 10.1016/j.jsbmb.2019.03.016
- [15] Kozhemiakin, Yu. M., Khromov, O. S., Filonenko, M. A., & Saifetdinova, G. A. (2002). *Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними [Scientific and Practical Recommendations for Keeping Laboratory Animals and Working with Them]*. Київ: Авіцена - Kyiv: Avicenna.
- [16] Lapach, S. N., Gubenko, A. V., & Babich, P. N. (2000). *Статистические методы в медико-биологических исследованиях с использованием Excel [Statistical Methods in Biomedical Research Using Excel]*. Киев: Морион - Kiev: Morion.
- [17] Livingston, M., Kalansooriya, A., Hartland, A. J., Ramachandran, S., & Heald, A. (2017). Serum testosterone levels in male hypogonadism: Why and when to check - A review. *International Journal of Clinical Practice*, 71(11), e12995. doi: 10.1111/ijcp.12995
- [18] Maghsoumi-Norouzabad, L., Zare Javid, A., Mansoori, A., Dadfar, M., & Serajian, A. (2021). The effects of Vitamin D3 supplementation on Spermatogram and endocrine factors in asthenozoospermia infertile men: a randomized, triple blind, placebo-controlled clinical trial. *Reproductive Biology and Endocrinology*, 19(1), 1-16. doi: 10.1186/s12958-021-00789-y
- [19] Nasreen, K., Ishrat, S., Banu, J., Fatima, P., Ansary, S. A., Jahan, I., ... & Islam, M. K. (2021). Comparison of vitamin D (25OHD) status between fertile and infertile men. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 10(4), 1303-1310. doi: 10.18203/2320-1770.ijrcog20211104
- [20] O'Shaughnessy, P. J. (2014). Hormonal control of germ cell development and spermatogenesis. In *Seminars in Cell & Developmental Biology*, 29, 65. doi: 10.1016/j.semcdb.2014.02.010
- [21] Ostrovska, S. S., Abramov, S. V., Pisarevska, I. A., Trushenko, O. S., Zherzhova, T. A., Pervyshester, K. Yu., ... & Zakharyev, A. V. (2021). Токсичний вплив кадмію на репродуктивну функцію чоловіків [Toxic effect of cadmium on male reproductive function]. *Український журнал медицини, біології та спорту - Ukrainian Journal of Medicine, Biology and Sports*, 6(6/34), 275-281. doi: 10.26693/jmbs06.06.275
- [22] Punab, M., Poolamets, O., Paju, P., Vihljajev, V., Pomm, K., Ladv, R., ... & Laan, M. (2017). Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. *Human Reproduction*, 32(1), 18-31. doi: 10.1093/humrep/dew284
- [23] Reznikov, O. H. (2003). Загальні етичні принципи експериментів на тваринах [General ethical principles of animal experiments]. *Ендокринологія - Endocrinology*, 8(1), 142-145.
- [24] Smolienko, N. P., Korenieva, E. M., Marakhovskiy, I. O., Chistyakova, E. E., Velichko, N. F., Bielkina, I. O., & Bondarenko, V. O. (2022). Статева поведінка самців щурів із гонадопатією після корекції вітаміном D3 та Трібестаном [Sexual behavior of male rats with gonadopathy after correction with vitamin D3 and Tribestan]. *Вісник проблем біології і медицини - Herald of Problems of Biology and Medicine*, 2(1/164), 240-248. doi: 10.29254/2077-4214-2022-2-1-164-240-248
- [25] World Health Organization. (2018). International Classification of Diseases, 11th Revision (ICD-11). Geneva: Switzerland.
- [26] Zaichenko, H. V., Yakovleva, L. V., Butenko, I. G., & Laryanovskaya, Yu. B. (2008). Вплив глюкозаміну гідрохлориду на сперматогенез щурів при його порушенні серотоніном [Effect of glucosamine hydrochloride on spermatogenesis in rats when it is disturbed by Serotonin]. *Фармакологія та лікарська токсикологія - Pharmacology and Medicinal Toxicology*, 1(3), 74-79.

МОРФОЛОГІЧНА БУДОВА СІМ'ЯНИКІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ГОНАДОПАТІЇ ТА ПІСЛЯ ЗАСТОСУВАННЯ ХОЛЕКАЛЬЦИФЕРОЛУ У КОМПЛЕКСНИХ СХЕМАХ КОРЕКЦІЇ

Мароховський І. О., Коренєва Є. М., Лар'яновська Ю. Б., Смоленко Н. П., Белкіна І. О., Величко Н. Ф., Чистякова Е. Є., Місюра К. В., Бондаренко В. О.

Проблема чоловічого безпліддя є актуальною та потребує вирішення. Застосування вітаміну D₃ (віт. D₃) у схемах лікування безпліддя може позитивно впливати на репродуктивну здатність особин чоловічої статі. Мета даного дослідження - визначити вплив вітаміну D₃, застосованого самостійно або разом із препаратом, що містить екстракт яєць сланких, на зміни гістологічної картини морфологічної будови сім'яників щурів із експериментальною гонадопатією. Самців щурів зі змодельованою патологією репродуктивної функції (серотоніновою гонадопатією) розподіляли на групи, котрі отримували корекцію за допомогою холекальциферолу окремо або разом із референтним препаратом Трібестаном (Тр), що містить екстракт яєць сланких. На зрізах сім'яників (забарвлення гематоксиліном та еозином) окрім оглядової мікроскопії проводили морфометричну оцінку сперматогенезу. Статистичну обробку проводили в стандартному пакеті програм "Statistica 6.0" з використанням t-критерію Ст'юдента та непараметричного аналогу однофакторного дисперсійного аналізу - критерій Краскела-Уоліса, після чого застосовували критерій Мана-Уїтні. Встановлено, що сім'яні каналці сім'яників щурів зі серотоніновою гонадопатією виразно зменшені у розмірі, власна їхня оболонка потовщена. Клітини Сертоллі часто деструктивно змінені, порушена рівномірність їх розташування. Спостерігалось зниження маси гонад, над'ячка, передміхурової залози та гіпоандрогенізація, знижувалися показники сперматогенезу. Після введення на тлі патології вітаміну D₃ відмічалось покращення морфологічної картини гонад. Сім'яні каналці мали нормальну гістологічну структуру. Статеві клітини розташовувалися концентричними рядами у відповідності до стадій розвитку, клітини Сертоллі візуально були незміненими. Популяція клітин Лейдига візуально більш гетерогенна, ніж у контрольних тварин. Однак, іноді виявлялися сім'яні каналці з вогнищевим некробіозом статевих клітин та дистрофією клітин Сертоллі, редукцією рядів статевих клітин. В цілому кількісні показники процесу сперматогенезу після введення вітаміну D₃ покращилися порівняно зі щурами з експериментальною гонадопатією, хоча і не досягали показників інтактного контролю. Введення на тлі гонадопатії комбінації

вітаміну D_3 та Трібестану потенціювало позитивний ефект кожного з компонентів при монозастосуванні. У щурів повністю відновлено мікроскопічний стан тестикулярної тканини. Сумісне використання вітаміну D_3 з препаратом Трібестаном нормалізувало масу гонад, їхніх придатків, значно зменшувало на гістологічних зрізах дистрофічно-деструктивні прояви у статевих клітинах у період росту і диференціювання, покращувало відносний рівень андрогенного статусу організму, позитивно впливало на сперматогенез у гонадах. Таким чином, нами встановлено, що комбіноване використання холекальциферолу та препарату Трібестан для корекції експериментальної гонадопатії проявляло більш ефективну дію, ніж використання одного із зазначених компонентів.

Ключові слова: гістологічна будова сім'яника, гонадопатія, вітамін D, холекальциферол, сперматогенез.
