Influence of vitamin D on the histostructure of the testis and morphometric indications of spermatogenesis of intact rats


One of the current problems is the study of the effects of vitamin D on the body, and in men its action is closely related to the pathogenesis of androgen deficiency and hypofertility. Particular attention needs to be paid to determining whether cholecalciferol (D3) has a negative effect on the gonads and spermatogenesis of intact individuals, as vitamin D therapy is used in reproductive disorders with or without vitamin D deficiency. The aim of the study was to determine the effect of vitamin D on the histological structure of gonads and morphometric parameters of spermatogenesis of adult intact male rats. The studies were performed on adult sexually active male Wistar rats. Vitamin D3 was administered orally in doses of 1000 IU, 4000 IU and 10000 IU. The solutions were made on seed oil. The control was intact rats. Vitamin D and its solvent were administered throughout the period of spermatogenesis and the time of maturation of sperm in the epididymis, after which the structural organization of the testes was determined. Gonadal samples were fixed in 10 % formalin solution, leave in alcohols of increasing strength, and embedded in paraffin. In addition to survey microscopy, morphometric evaluation of the process of spermatogenesis was performed on sections of gonads stained with hematoxylin and eosin. Micropreparations were examined using a Granum L 30 (03) light microscope, and microscopic images were taken with a Granum DSM 310 digital video camera. Photographs were processed on a Pentium 2.4 GHz computer using Toup View. Statistical processing of data results was performed in the standard software package "Statistica 6.0" using Student's t-test and using a non-parametric analogue of one-way analysis of variance - Kruskal-Wallis criterion. In rat testicular sections, seminiferous tubules were located transversely or obliquely and were oval or round in shape. The diameter of the tubules is normal, the tubular membrane, as well as the protein and vascular membranes were normal. The basal department contains the youngest cells of the germinal epithelium - spermatogonia. Cells have a pronounced functional activity. Morphometric characteristics of spermatogenesis of intact rats corresponded to the physiological norm. The introduction of the solvent throughout the period of spermatogenesis and maturation of mature sperm in the epididymis did not affect the histoarchitectonics of the testicles. The testicular lobes are filled with concentric or flattened profiles of sections of the seminal tubules, which are close enough to each other. The diameter of the tubules is normal, the intrinic membrane of the tubules, as well as the protein and vascular membranes corresponded to those in intact animals. 3-4 generations of spermatogenic cells, which were at different stages of development, can be seen in the tubules. However, few tubules with focal destruction of the germinal epithelium and exfoliation of germ cells in the lumen of the tubules have been observed. No significant changes in the microstructure of the seminal tubules were observed after administration of vitamin D at all doses studied. Not only spermatogenesis but also spermiogenesis is clearly traced in different tubules of rats - stages of cellular transformations from spermatid to sperm. Morphometric parameters of the process of spermatogenesis of rats receiving different doses of vitamin D3 do not differ from those of intact rats. Thus, the use of vitamin D in these doses revealed the safety of its effect on the number of spermatogonia and tubules with stage 12 meiosis. When cholecalciferol was used in male rats for 68 days, the
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**Introduction**

One of the current problems is the study of the effects of vitamin D on the body [11], and in men its action is closely related to the pathogenesis of androgen deficiency and hypofertility [17]. Seasonal fluctuations in vitamin D levels have been found, with high levels in summer and autumn and low levels in winter and spring, which are almost identical to similar annual cycles of testosterone levels, suggesting a strong association between androgen metabolism and vitamin D [22].

It has been shown that in men the level of vitamin D determines the qualitative and quantitative parameters of ejaculate [6, 16], including motility and morphology of sperm [1, 14]. There is growing evidence of the importance of vitamin D for sperm maturation. An association between low levels of vitamin D and decreased numbers of motile and morphologically normal sperm has been reported [10].

Vitamin D deficiency in animals leads to impaired maturation of the vas deferens, decreased testicular weight and sperm concentration [8]. The experiment showed that vitamin D saturation leads to a significant improvement in spermatogenesis in experimental reproductive diseases [12, 21]. Evidence from the literature on the use of vitamin D on the male body to demonstrate the wide variability in design, methodology, patient population, reference values and routes of administration of both vitamin D and its metabolites and requires further study.

Vitamin D belongs to the group of fat-soluble secosteroids. Secosteroid is a molecule that is very similar to steroids, but with a broken steroid ring. Vitamin D is naturally available in several forms, but only two forms are important for the human body (D2 and D3), which differ chemically in their side chains. These structural differences alter their binding to the carrier protein, ie vitamin D-binding protein, and their metabolism, but in general the biological activity of these active derivatives is close [7, 15].

Particular attention needs to be paid to determining whether cholecalciferol (D3), which is the most active metabolite of this vitamin and is often used in the clinic, has an adverse effect on gonads and spermatogenesis of intact individuals, as vitamin D therapy is used in reproductive disorders vitamin D deficiency, so without it [4].

The aim of the study was to determine the effect of vitamin D on the histological structure of gonads and morphometric parameters of spermatogenesis of adult intact male rats.

**Materials and methods**

The study was performed on adult sexually active male Wistar rats weighing 250-300 g, kept in standard vivarium conditions under natural light and drinking mode ad libitum in accordance with the national "General Ethical Principles of Animal Experiment" (Minutes № 2 meeting of the Bioethics Commission from January 29, 2021).

Vitamin D3 was administered orally in doses of 1000 IU, 4000 IU and 10000 IU. The solutions were made on seed oil from vitamin D3 (powder) (China, batch CHG20062009, which meets the quality standard GB 9840-2017). The control was intact rats. Vitamin D in various doses and its solvent were administered throughout the period of spermatogenesis and the time of maturation of sperm in the epididymis - only 68 days. At the end of the introduction of animals was removed from the experiment by decapitation under mild chloroform anesthesia.

After euthanasia, rats' gonads were removed and weighed, structural organization of the testes was further studied. Gonadal samples were fixed in 10 % formalin solution, in alcohols of increasing strength, and embedded in paraffin. In addition to survey microscopy, morphometric evaluation of the process of spermatogenesis was performed on sections of gonads stained with hematoxylin and eosin [2].

Micropreparations were examined using a Granum L 30 (03) light microscope, and microscopic images were taken with a Granum DCM 310 digital video camera. Photographs were processed on a 2.4 GHz Pentium computer using Toup View.

Statistical processing of data results was performed in the standard software package "Statistica 6.0" using Student's t-test and using a non-parametric analogue of one-way analysis of variance - Kruskal-Wallis criterion, followed by the Mann-Whitney test [13].

**Results**

The testicles of intact rats served as a general intact control in our study. Figure 1 and Table 1 show the histostructure of testicular tissue and morphometric parameters that characterize the process of spermatogenesis of these animals. Light microscopy showed that the testicles of rats, which did not receive any substances, convoluted seminal tubules were cut in the transverse or oblique direction and had an oval or round shape. The diameter of the tubules is normal, the tubule membrane, as well as the protein and vascular membranes were normal [9]. The wall of the seminal tubules is built of germ cells. The basal department contains the youngest cells of the germinal epithelium - spermatagonia. Among them are cells with chromatin in the nucleus of condensed (type B) and non-condensed spermatogonia index remained at the level of animals that did not receive the test substance. The use of vitamin D3 in intact adult male rats does not adversely affect the histological structure of the testes.

**Keywords:** vitamin D, testicle, gonadotoxicity, testicular histostructure, morphometric parameters of spermatogenesis.
(type A) species. Spermatogonia type A is represented by both so-called light (renewable) and dark (reserve) cells.

Mitosis is sometimes seen in the spermatogonia of intact animals. Spermatocytes are located in the intermediate part of the wall of the seminiferous tubule. Most of the first-order spermatocytes were in the pachynema stage. Metaphases of the first and (much less often) second division and anaphases of these divisions were well observed in some tubules. Numerous spermatids and formed spermatozoa are observed in the adluminal compartment of the seminiferous tubules. Germ cells of different stages of development are arranged in a strict order, concentric layers according to the stages of the spermatogenic cycle. The associations of germ cells are clearly demarcated, in different tubules, but also spermiogenesis - stages of cellular transformations from spermatid to sperm. The germinal epithelium tape contained at least 4-6 rows of cells. Numerous Sertoli cells are located between the spermatogonia on the basement membrane. Their light pear-shaped nucleus with a nucleolus is clearly visible. Cytoplasmic processes of cells are masked by germ cells of the subsequent stages of development. Interstitial connective tissue is very limited. Clusters (up to 7-20) of Leydig cells and a few fibroblasts are visible near the blood vessels contained in the intertubular loci. The nuclei were mostly round-oval in shape, rather large in volume, well-contoured, normochromic, with a noticeable uniform "scattering" of chromatin granules, and contained mostly one nucleolus. The cytoplasm of cells is intensely eosinophilic, in single cells on the periphery is weakly vacuolated, cell boundaries are blurred. The described state of Leydig cells characterizes the normal functional

**Table 1. The effect of vitamin D on the quantitative indicators of spermatogenesis process in rats.**

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>The amount of normal spermatogonia in the tubule, (M±m)</th>
<th>Number of tubules with stage 12 meiosis, %, Me (LQ; UQ)</th>
<th>The number of tubules with squamous epithelium, %, Me (LQ; UQ)</th>
<th>Index of spermatogenesis, points, Me (LQ; UQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>61.07±0.5</td>
<td>4.0 (3.0; 5.0)</td>
<td>0 (0; 0)</td>
<td>3.33 (3.32; 3.34)</td>
</tr>
<tr>
<td>Solvent</td>
<td>61.23±0.66</td>
<td>2.5 (2.0; 4.0)</td>
<td>0.0 (0; 3.0)</td>
<td>3.30 (3.26; 3.30)</td>
</tr>
<tr>
<td>Vitamin D3, 1000 IU</td>
<td>60.89±13.0</td>
<td>4.0 (3.0; 5.0)</td>
<td>0.0 (0; 1.0)</td>
<td>3.34 (3.33; 3.34)</td>
</tr>
<tr>
<td>Vitamin D3, 4000 IU</td>
<td>61.23±0.77</td>
<td>3.0 (3.0; 4.0)</td>
<td>0 (0; 0)</td>
<td>3.33 (3.33; 3.34)</td>
</tr>
<tr>
<td>Vitamin D3, 10000 IU</td>
<td>61.41±0.34</td>
<td>3.00 (3.0; 4.0)</td>
<td>0 (0; 1.0)</td>
<td>3.33 (3.32; 3.34)</td>
</tr>
</tbody>
</table>

**Notes:** Me - median; LQ - lower quartile; UQ - upper quartile.
activity of cells.

Morphometric characteristics of spermatogenesis of intact rats corresponded to the physiological norm for these animals (see Table 1).

The introduction of the solvent throughout the period of spermatogenesis and maturation of mature sperm in the epididymis did not affect the histoarchitectonics of the testicles. The testicular lobes are filled with concentric or flattened profiles of sections of the seminal tubules, which are close enough to each other. The diameter of the tubules is normal, the tubular membrane, as well as the protein and vascular membranes were normal. 3-4 generations of spermatogenic cells, which were at different stages of development, can be seen in the tubules. The cells are arranged in concentric layers according to the stages of the spermatogenic cycle. In all rats of this group, the cell population is presented in full number. However, in one animal, against the background of most unchanged seminal tubules, a few tubules were observed with focal destruction of the germinal epithelium, looseness of the rows, exfoliation of germ cells in the lumen of the tubules (Fig. 2).

Quantitative indicators characterizing the process of
spermatogenesis of rats injected with seed oil are given in the above table 1.

No significant changes in the microstructure of the seminal tubules were observed after administration of vitamin D at doses of 1000 IU, 4000 IU and 10000 IU. In different tubules in rats clearly traced not only spermatogenesis (the process of successive rearrangements of germ cells: spermatogonia - sperm), but also spermiogenesis - stages of cellular transformation from spermatids to sperm (Fig. 3).

Quantitative indicators of the process of spermatogenesis of rats receiving different doses of vitamin D3 are presented in table 1.

**Discussion**

Drugs can affect reproductive function, causing not only ugliness or death of embryos and fetuses, but also disrupting gametogenesis and preventing fertilization [19]. Therefore, when evaluating pharmacological substances in their safety studies, their effect on reproductive function should be investigated in animal studies [1], part of this study was the study of the safety of cholecalciferol (vitamin D3) on testicular histostructure and morphometric parameters of spermatogenesis of intact rats. Detection of gonadopathies in animals requires special experimental approaches, which is why we studied three doses and long-term intake of the substance to identify the effects of gonadal lesions, which may later manifest themselves in inability or reduced ability to conceive or in fetal development, and also in subsequent generations.

In the study of the testes of intact adult rats, we observed not only the normal structure of germinal epithelium cells, Sertoli cells, but also Leydig cells, which characterizes their functional activity [20], led to the normal course of spermatogenesis, was reflected in morphometric parameters and corresponded to the physiological norm for these animals.

The safety of long-term intake of cholecalciferol in relation to the histological picture of male gonads was studied at a dose of 1000 IU, which is ten times greater than it, and conditionally therapeutic. In the study of the effect of vitamin D not only in the minimum dose, therapeutic but also in high (10000 IU) morphological structure of testes and morphometric parameters of spermatogenesis did not differ from that of intact animals, ie corresponded to the data obtained in rats of appropriate age [5, 19]. Considering that only quantitative assessments in the safety of drugs are evidence to establish the level of harmful gonadotoxic effects of drugs, pathomorphological morphometric methods are used to determine the degree of disorders that occurred in the gonads [1].

The number of normal spermatogonia in the tubule, the proportion of tubules with stage 12 meiosis and squamous epithelium probably did not differ between the studied groups with different dosage of the test substance and morphometric parameters of intact rats, which also indicates harmlessness to germinal epithelium. The relative number of different populations of seminiferous tubules - the index of spermatogenesis, which does not change with the studied doses of vitamin D, suggests the possibility of its use for a long time in individuals who did not have reproductive disorders, given that cholecalciferol is usually prescribed long courses, and recently in high doses [18].

Reproductive safety studies in males are studied after chronic administration, as different generations of the epithelial epithelium are equally sensitive to chemical agents. In this regard, only the repeated action of the test compound at all stages of spermatogenesis allows to detect pathology [1]. Animal administration of cholecalciferol in all three doses for almost seventy days did not lead to disruption of gonads and spermatogenesis, which may indicate its safety against gonads under these conditions. Under the action of solvent (seed oil) signs of focal destruction of germinal epithelium were observed in only one male rat, in general, they do not significantly affect both the histological picture of gonads and general morphometric parameters of spermatogenesis.

Thus, it can be stated that vitamin D3 when used in intact individuals in the studied doses does not have a negative effect on the youngest cells of the germinal epithelium - spermatogonia; does not reduce the number of tubules with stage 12 meiosis - ie does not reduce the reserve of spermatogenesis. As a result, the index of spermatogenesis does not change.

The data obtained for the first time show the safety of vitamin D3 in terms of testicular morphological structure and spermatogenesis, which was studied by morphometric studies of structural elements of the gonads, and are of great practical importance because they indicate the safety of cholecalciferol in both minimum and even maximum doses [18] under the conditions of modeling vitamin D deficiency.

**Conclusion**

1. When using cholecalciferol in doses of 1000 IU, 4000 IU and 10000 IU, the safety for both spermatogonia and tubules with stage 12 meiosis were confirmed.

2. When cholecalciferol was used in adult rats for 68 days, the spermatogenesis index remained at the level of animals that did not receive the test substance or diluent.

3. The use of vitamin D3 in intact mature male rats does not adversely affect the histological structure of the testes.

**References**


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слугували інтактні щури. Вітамін D та його розчинник вводили впродовж всього періоду сперматогенезу і часу дозрівання сперматозойдів у придатку сім'яника, після чого визначали структурну організацію сім'яни. Зразки статевих залоз фіксували у 10% розчині формаліну, проводили по спинках міцності, що зростає, та заликали у парафін. На зрізах зображень здійснювали цифрову відеокамерою Granum DCM 310. Фотознімки обробляли на комп'ютері Pentium 2,4 GHz за допомогою програми Tour View. Статистичну обробку результатів даних проводили в стандартному пакеті програм "Statistica 6.0" з використанням t-критерію Стьюдента та застосовуючи непараметричний аналог однофакторного дисперсійного аналізу - критерій Краскела-Уоліса. На зрізах сім'яників інтактних щурів звивисті сім'яні канальці були розташовані у поперечному або косому напрямку і мало свільну чи округлу форму. Діаметр каналів звичайний, епителиальна оболонка канальців, а також білкова та судинна оболонки відповідали нормі. В базальному відділі містяться найбільш молоді клітини сперматогенного епітелію - сперматогонії. Клітини мають виражену функціональну активність. Морфометрична характеристика сперматогенезу інтактних щурів відповідала фізіологічній нормі. Введення розчинника протягом всього періоду сперматогенезу та дозрівання зрілих сперміїв у придатку сім'яника не вплинуло на гістоархітектоніку тестикул. Часточки сім'яника заповнені концентричними або сплощеними профілями зрізів сім'яних каналів, які досить щільно прилягали один до одного. В каналіцах звичайний, епителіальні оболонки сім'яних каналів, а також білкова та судинна оболонки відповідали таким у інтактних тварин. В каналіцах видно 3-4 генерації сперматозойдів, що знаходилися на різних стадіях розвитку. Однак простежено нечисленні каналі з вонищею деструкцією епітеліосперматогенного пласта та зруйнуванням старих клітин. В каналіцах сперматогенез був відповідний фізіологічній нормі. Введення вітаміну D у всіх вивчених дозах не вплинуло на гістологічну картину сім'яника. На зрізах сім'яника звивистих сім'янних каналів були зруйновані у поперечному або косому напрямку. Діаметр каналів звичайний, епителиальна оболонка каналів, а також білкова та судинна оболонки відповідали таким у інтактних тварин. В каналіцах сперматогенез був відповідний фізіологічній нормі. Введення вітаміну D у всіх вивчених дозах не вплинуло на гістологічну картину сім'яника. Ключові слова: вітамін D, сім'яник, гонадотоксичність, гістоструктура сім'яників, морфометричні показники сперматогенезу.

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