

ЕКСПЕРИМЕНТАЛЬНІ ДОСЛІДЖЕННЯ

SEXUAL BEHAVIOUR OF THE INTACT RAT MALES UNDER THE CONDITION OF EXOGENOUS CHOLECALCIFEROL INTAKE*

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The sexual behaviour plays an important role in the reproduction process; it is instinctive and is striking in its conservatism and stereotypes. However, the reproductive system is considered to be the most vulnerable [1], because of its dependence from different psychological and organic factors which may disturb the erectile function, may affect the timeliness of ejaculation, the delaying of which in some cases can be the reason of infertility [2].

Inability to achieve an erection affects the men around the world. The spreading of this global problem ranges from 13 % to 71 % in certain countries [3]. The vascular and neurogenic factors are the most often reason of erectile dysfunction [4]. The neurogenic factors may cause the development of erectile dysfunction at the all levels of nervous system. The

vascular form of erectile dysfunction manifests mainly at local level by causing the atherosclerosis of arteries which disturbs the peripheral blood circulation and reflects the changes of penis bloodstream.

There are scientific researches which confirm some vitamins can prevent or relieve symptoms of sexual disturbances. The deficiency of this vitamins may cause the disorders of reproductive function and, in particular, sexual behaviour [5]. The well-researched at first sight, oil soluble secosteroid — vitamin (hormone) D, especially its the most active form — D₃ (cholecalciferol) more and more often draws attention of the researchers. There are reports about link between reproductive function and vitamin D blood level [6, 7], which can affect the sexual behaviour [3, 8]. The regulation of

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endothelial function is the main mechanism of vitamin D activity which helps this vitamin to affect the sexual function [9]. The lack of vitamin D causes endothelial dysfunction in men hindering nitrogen oxide releasing and blood filling of vessels which are necessary for erection to be supported and sexual encounter to be carried out [10]. Vitamin D deficiency even in the prenatal and postnatal periods is the important cause of risk of erectile dysfunction developing in adulthood [11]. A lot of researchers have shown the increasing of number of cases of erectile dysfunction in men

The majority of researches have shown the number of erectile dysfunction is increased over time on the down stages of ontogenesis.

MATERIALS AND METHODS

The investigation had been carried out in accordance with the National «General Principles for Animal Research Ethics» (Ukraine, 2001), which corresponds to the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasburg, 1985).

The sexual behaviour has been studied in sexual active 7-month-old Wistar rats' males with average weight of 250-300 g which were kept under the standard condition of vivarium, in cages separately, on the 58th day of the introduction of vitamin D₃. The solution of vitamin D₃ has been introduced in volume of 0.5 ml in the doses of 1000 IU (Vit.D₃-1000 group), 4000 IU (Vit. D₃-4000 group) and 10000 IU (Vit. D₃-10000 group). Control group of animals has received Apricot kernel oil as a solvent. The solutions of vitamin D₃ were prepared using apricot kernel oil and vitamin D₃ substance (China, Lot. CHG20062009, quality standard GB 9840-2017).

RESULTS AND THEIR DISCUSSION

The sexual behaviour of rat males of Control group has been characterized by evidence of courting with some quantity of sniffing of females' perineum (Table 1).

The mountings in males have been detected within 1.5 minutes after contacting with females. The signs of mating behaviour — intromissions — were observed within 3 minutes in average of each group. Only 40% of animals

The link between erectile dysfunction and D hypovitaminosis is age depended [8]. However, the literature sources about vitamin D ability to increase testosterone level which is lowering with age and in this way to improve the sexual activity are controversial [12].

In spite of current scientific developments, the safety of application of different doses of vitamin D used for reproductive pathologies treatment and correction of sexual function remains questionable and needs to be clarified. That why, the *aim* of our investigation is to study the altering of sexual behaviour of intact rat males under the condition of exogenous intake of vitamin D₃ in different doses (1000 MO, 4000 MO, 10000 MO).

The sexual behaviour has been studied during mating with ovariectomized receptive females during 15 minutes in dark period of time according to the quantitative and time indices of mountings, intromissions and ejaculations. The receptivity of ovariectomized females was achieved by consistent introduction of Estradiol dipropionate oil solution (10 mkg per animal) 48 hours before and Progesterone (500 mkg per animal) 4–5 hours before testing.

The statistical analysis has been carried out using Shapiro-Wilks test to evaluate whether data obtained are normal distributed. The significant of differences between mean has been evaluated by Student t-test. Data were performed as arithmetic mean (\bar{X}) and its Standard deviations ($\pm S_{\bar{X}}$). The differences were considered to be significant at $P < 0.05$. χ^2 criterion was used for comparative analysis of relative values.

have achieved the ejaculation during the test. (Fig. 1; $p < 0.05$).

The evaluation of results has shown the sexual behaviour of rats received vitamin D₃ in dose of 1000 IU (vit. D₃-1000 group) hasn't statistically differed from control group (see Table 1, Fig. 1) Therefore, this dose of vitamin D₃ hasn't influenced the researched parameters.

Table 1

Quantitative indices of males sexual behaviour, $\bar{X} \pm S_{\bar{x}}$

Parameter	Group			
	Control, n = 5	Vit. D ₃ -1000 n = 8	Vit. D ₃ -4000 n = 8	Vit. D ₃ -10000 n = 8
Number of mountings	3.0 ± 0.5	4.1 ± 1.0	7.9 ± 1.1 ¹	6.4 ± 0.8 ¹
Number of intromissions	7.6 ± 0.5	5.4 ± 1.0	7.1 ± 1.1	18.6 ± 1.9 ¹
Number of ejaculations	0.4 ± 0.3	0.3 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Sniffing of female	9.2 ± 1.0	7.9 ± 0.5	13.4 ± 0.8 ¹	6.3 ± 0.5 ¹

Note:

¹⁾ statistically significant differences from Control group (p < 0.05).

The introduction of 4000 IU of vitamin D₃ has led to increase the contacts between males and females by 50% and the number of mountings by 2.3 times to compare to Control group (p < 0.05). This indicates the altering of males sexual behaviour which is regulated at central level. The half of animals of this group have demonstrated the accomplishing the sexual act which was evidenced by the onset of ejaculation. However, it wasn't statistically differed from control group.

The signs of altering of patterns of sexual behaviour regulated at central level have been detected in group of rats received 10000 IU of cholecalciferol: the declining of the contacts between males and females by 1.5 times (p < 0.05) and increasing of mountings number by 2.1 times (p < 0.05) in compare to Control group have been observed. At the same time the differences in the indices which is regulated by peripheral mechanisms of sexual behaviour have been detected in group received 10000 IU of vitamin D₃: the latent period of in-

tromissions has reduced by 2.1 times (p < 0.05) and the total number of intromissions has increased. The number of animals which have achieved ejaculation hasn't differed from indices of Control group and was 38%.

As it is known and according to our previous investigations [13], the acquisition of sexual skills is important for successful accomplishing of copulative cycle which creates stereotypical reaction in males and makes the indices steady. Therefore, the indices of 4th testing were considered to be the initial data. The rats' males which have achieved ejaculation during investigation have been considered to be sex active and were taken into experiment. These males consist 83% of 35 experimental animals.

The ability to commit a sexual act depends on sex hormones level. The levels of these hormones undergo seasonal and daily fluctuation and may change when the sex partner is close [14]. The steady indices of mating behaviour and copulation in experimental males may be explained by adequate level of androgens which

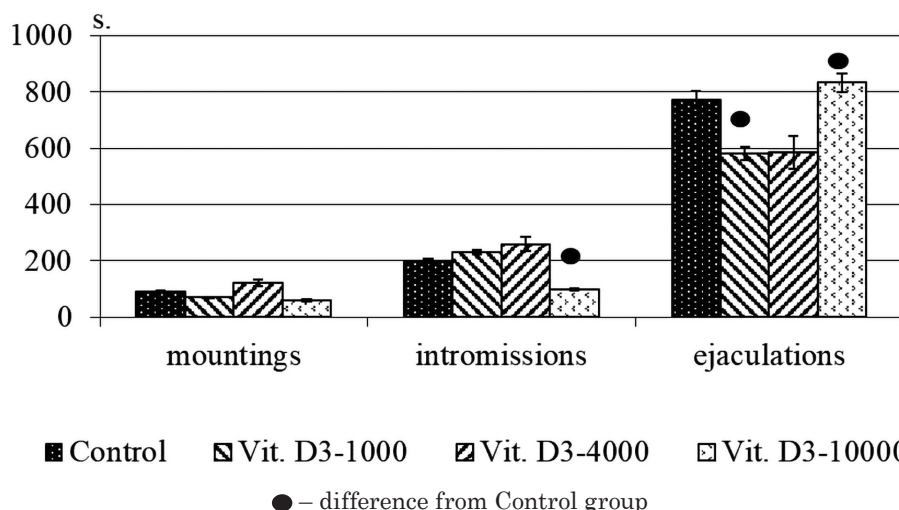


Fig. 1. Time indices of sexual behaviour of experimental animals.

were confirmed by normal structure of testicles in males received cholecalciferol [15], and such laboratory value as testosterone concentration which regulates NO production and vascular stiffness [9].

When estimating the data obtained, we proceeded from the fact that double mechanism of stimulation — central and peripheral, is necessary for adequate sexual behaviour [16]. Central mechanism is used for activation of mating behaviour in rodent (libido is the men' analogue). The regulated centers are situated in the anterior hypothalamus, when neurotransmitters and testosterone, which is converted into estradiol, are operating. The mating behaviour, mountings and postejaculatory interval are regulated by central mechanism. The peripheral mechanism which is necessary for copulation requires dihydrotestosterone. Testosterone through its metabolites — estradiol and dihydrotestosterone, initiates all the elements of sexual activity in males. Peripheral mechanism manifests in the intromission supporting, latent period of ejaculation and their number during the experiment. The regulation of the number of ejaculation is carried out on both levels. The integrative process of sex function controlled by two mechanisms (central — motivating and peripheral — copulatory) is coordinated on different levels with the involvement of neuronal structures, neuromediators and neuroendocrine systems. The implementation of sexual behaviour is regulated by central nervous system and is influenced by neurotransmitters which play the leading role in the central control of the hypothalamic-pituitary-gonad system [17].

The intake of 10000 IU of vitamin D₃ leads to the acceleration of mating and increasing of copulations which may be induced by increasing of concentration of intratesticular testosterone, dysfunction of steroid genesis or changing of hormonal and other ways of sex behaviour regulation which play, at least, the same important role [18, 19] and, probably, reveal the ambiguous role of vitamin D in the different processes of sexual function [9, 20].

Our previous investigation has shown that under the condition of prolonged using of vitamin D₃ in dose of 10000 IU the quality of sexual behaviour hasn't impacted the fertility and fecundity, but the declining of reproductive capacity of males has been observed. However, the histological samples of testicles of these animals haven't detected negative signs [15]. These investigations have proven the ambiguous influence of vitamin D₃ on the reproductive function.

The results of this work together with literature data fit the overall concept of vitamin D impact on the reproductive system in general and sexual behaviour in particular.

It is well known, that advisability of the usage of chosen doses is determined by signs detected in animals. During experiment the external appearance of animals, general and emotional condition remain without changes. Our investigation has demonstrated the harmlessness and safety of usage of chosen doses of vitamin D₃ as for sexual behaviour of rats' males. However, all listed benefits and possible negative consequences for reproductive function during correction of sexual behaviour disorders should be taken into account.

CONCLUSION

1. Exogenous long-term supplementation of vitamin D₃ in dose of 1000 IU to rats hasn't impacted the males' sexual behaviour.
2. The mating components of sexual behaviour have increased in intact males when vitamin D in dose of 4000 IU was used.
3. The using of vitamin D₃ in dose of 10000 IU in rat males has impacted the indices which are regulated on the central as well as peripheral mechanisms of sexual behaviour: the acceleration of mating and number of copulations have increased with fewer quantity of mating indices.

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The investigation of the impact of different doses of vitamin D₃ on the sexual behaviour is the solving current problem of sexual function disorders in men and discovering the new data about vitamin D₃ properties.

The aim of this work was to study the altering of sexual behaviour of intact rats' males under the condition of exogenous intake of different doses (1000 IU, 4000 IU, 10000 IU) of vitamin D₃.

Materials and methods. The sexual behaviour has been studied according to the standard method in sexually active 7-month-old Wistar rats' males with average weight of 250–300 g, on the 58th day of the introduction of vitamin D₃. The solution of vitamin D₃ has been introduced in volume of 0,5 ml in the doses of 1000 IU (Vit. D₃-1000 group), 4000 IU (Vit. D₃-4000 group) and 10000 IU (Vit. D₃-10000 group). Control group of animals has received Apricot kernel oil as a solvent.

The significant of differences between mean has been evaluated by Student t-test. The differences were considered to be significant at $p < 0.05$. χ^2 criterion was used for comparative analysis of relative values.

Results. Exogenous long-term supplementation of vitamin D₃ in dose of 1000 IU to rats hasn't impacted the males' sexual behaviour. The introduction of 4000 IU of vitamin D₃ has led to increase the contacts between males and females by 50% and the number of mountings by 2.3 times to compare to Control group ($p < 0.05$). This indicates the altering of males sexual behaviour which is regulated at central level. The signs of sexual behaviour regulated at central level have been detected in group of rats received 10000 IU of cholecalciferol: the declining of the contacts between males and females by 1.5 times ($p < 0.05$) and increasing of mountings num-

ber by 2.1 times ($p < 0.05$) in compare to Control group have been observed. The changing of sexual behaviour regulated at peripheral level have been detected: the latent period of intromissions was shorter by 2.1 times ($p < 0.05$) and their number has increased.

Conclusion. Exogenous long-term supplementation of vitamin D_3 in dose of 1000 IU to rats hasn't impacted the males' sexual behaviour, the mating indices have increased when vitamin D in dose of 4000 IU was used, the changes of all researched indices have been observed when vitamin D_3 in dose of 10000 IU was introduced.

Key words: vitamin D_3 , erectile dysfunction, rat males, sexual behaviour, cholecalciferol.

СТАТЕВА ПОВЕДІНКА ІНТАКТНИХ САМЦІВ ЩУРІВ ЗА УМОВ ЕКЗОГЕННОГО НАВАНТАЖЕННЯ ХОЛЕКАЛЬЦИФЕРОЛОМ

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Дослідження впливу різних доз вітаміну D_3 на сексуальну поведінку є вирішенням актуальної проблеми розладів статевої функції у чоловіків і відкриттям нових даних про властивості вітаміну D_3 .

Метою роботи було вивчення змін статевої поведінки інтактних самців щурів за умов екзогенного надходження різних доз (1000 МО, 4000 МО, 10000 МО) вітаміну D_3 .

Матеріали та методи. На сексуальноактивних семимісячних самцях щурів популяції Вістар масою 250–300 г була досліджена статеву поведінку на 58 добу введення вітаміну D_3 в дозах 1000 МО, 4000 МО та 10000 МО *per os* (в 0,5 мл розчину, який виготовляли з порошку) або розчинника, в якості якого застосовували кісточкову абрикосову олію. Вірогідність розбіжностей середніх величин визначали за *t* критерієм Стюдента. При порівняльному аналізі відносних величин використовували критерій χ^2 . Розбіжності вважалися значущими при $p < 0,05$.

Результати. Показано, що навантаження щурів холекальциферолом в дозі 1000 МО не веде до змін в статевій поведінці піддослідних щурів. При дозі 4000 МО призводить до збільшення в 1,5 раза ($p < 0,05$) кількості наближень самця до самки та в 2,3 раза ($p < 0,05$) кількості садок, що вказує на зміни у центральному механізмі статевої поведінки. При отриманні вітаміну D_3 в дозі 10000 МО спостерігались прояви, що регулюються на центральному механізмі статевої поведінки, які виражались у зменшенні в 1,5 раза ($p < 0,05$) кількості наближень самця до самки та збільшенні в 2,1 раза ($p < 0,05$) кількості садок, а також виявлялись зміни, що регулюються на периферійному рівні: скорочувався латентний період інтромісій в 2,1 раза ($p < 0,05$) та збільшувалась їх кількість.

Висновки. Екзогенне тривале навантаження щурів вітаміном D_3 в дозі 1000 МО не позначається на статевій поведінці самців, при дозі 4000 МО збільшуються залицяльні показники, при дозі 10000 МО спостерігаються зміни у всіх досліджених показниках статевої поведінки.

Ключові слова: вітамін D_3 , еректильна дисфункція, самці щурів, статеву поведінку, холекальциферол.