11. Дмитриева Л.А. Пародонтит. — М.: МЕД-пресс-информ, 2007 — 500 с. 12. Сивовол С.И. Первичные факторы в этиологии и патогенезе воспалительных заболеваний пародонта // Стоматолог. — 2006. — № 6. — С. 37-48.

12. Власов А.А., Малахов В.В., Николаева Н.Б., Сафронов А.А., Умникова М.В. «ДиаДЭНС». Руководство по динамической электронейростимуляции аппаратами ДиаДэнс-Т и ДиаДэнс-ДТ. Екатеринбург. – 2005. – 284 с.

13. Лукиных Л.М., Жулев Е.Н., Чупрунова И.Н. «Болезни пародонта. Клиника, диагностика, лечение и профилактика». Руководство. Издательство НГМА. Нижний Новгород. – 2005. – 322 с.

14. Лукиных Л.М., Успенская О.А. «Физиотерапия в практике терапевтической стоматологии» Нижний Новгород. – 2006. – 36 с. 4.

15. Орехова Л.Ю., Кучумова Е.Д., Стюф Я.В. и др. Основы профессиональной гигиены полости рта: методические указания – «Поли Медиа Пресс», С.-Петербург. – 2004. – 56 с.

16. Муравянникова Ж.Г. Основы стоматологической физиотерапии. Ростов-на-Дону, Феникс – 2003. – 320 с.

17. Мандра Ю.В.,Шимова М.Е.,Шнейдер О.Л.,Светлакова Е.Н.,Ваневская Е.А. Повышение эффективности комплексного лечения заболеваний пародонта с применением динамичесой электронейростимуляции. ГОУ ВПО УГМА Росздрава, г. Екатеринбург Терапевтическая стоматология , стр.21 https://cyberleninka.ru/article/n/povyshenie-effektivnosti-kompleksnogo-lecheniya-zabolevaniy-parodonta-s-primeneniem-dinamicheskoy-elektroneyrostimulyatsii/viewer

18. Мандра Ю.В.,Жегалина Н.М.,Светлакова и др.Эффективность применения динамической электронейростимуляции после лазерной гингивэктомии ГОУ ВПО УГМА Росздрава, г. Екатеринбург Терапевтическая стоматология , стр.15

19. Аббасова Р.А. и др.Применение аппарата ДиаДэнас в комплексном лечении альвеолита Молодой ученый № 14(148),2017

UDC: 612.83: 612.662.9: 618.173-073.7 / -076-085: 615.2.1-092.9

THE EFFECT OF TESTOSTERONE DEFICIENCY ON THE BIOELECTRICAL ACTIVITY OF THE EFFERENT LINK OF THE SOMATIC REFLEX ARC

Rodinsky O.
SU "Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine", the department of physiology. Head of department – prof. Rodinsky O.G. Ukraine, Dnipro, st. V. Vernadsky 9
Tkachenko S.
SU "Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine", the department of physiology. Head of department – prof. Rodinsky O.G. Ukraine, Dnipro, st. V. Vernadsky 9
Marazha I.
SU "Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine", the department of physiology. Head of department – prof. Rodinsky O.G. Ukraine, Dnipro, st. V. Vernadsky 9
Deforzh G.
Central Ukrainian State Pedagogical University named after Vladimir Vinnichenko. Ukraine, Kropyvnytskyi, vul. Shevchenka 1

Abstract

In conditions of prolonged hypoandrogenemia, changes in the bioelectric activity of the neuromuscular system were studied. The study was performed on 24 rats - males of the Wistar 5-6 months old, weighing 180-260 g. A model of the hypoandrogenic state was created by bilateral orchiectomy. After 120 days, the animals were involved in an acute experiment. Irritation (electrical impulses of a rectangular shape) was applied to the sciatic nerve, caused by the action potential was diverted from the calf muscle. We analyzed the threshold, chronaxy, latency, refractoriness (by applying double impulses), and the functional stability of the neuromuscular synapse by stimulation of the sciatic nerve with packs of 10 impulses. The muscle excitation threshold increased by 333.96 ± 2.17% with indirect and 41.67 ± 4.41% with direct irritation ($p < 0.01$). The latent period increased by 31.93 ± 2.55%, the amplitude of the response increased by 65.44 ± 5.71%, the duration of the action potential increased by 101.99 ± 2.54% compared with the control group ($p < 0.001$). For stimulation with paired stimuli, starting from the interval of 3 ms, a significant decrease in the rate of restoration of the amplitude of the response to the test stimulus in the group of animals with experimental hypoandrogenemia was revealed. During stimulation with bursts of impulses, starting from a frequency of 50 Hz, in animals with orchiectomy, a significant decrease in the amplitude of response caused by the tenth stimulus was observed. And at frequencies above 200 Hz, a rhythm transformation was observed.

Thus, in conditions of prolonged hypoandrogenemia, a pronounced decrease in the excitability of the neuromuscular apparatus occurs, moreover due to the nervous structures, against the background of a decrease in lability and an increase in the phenomenon of homosynaptic depression in the neuromuscular synapses.

Keywords: androgens, neuromuscular synapse, muscle, excitation

Introduction. Testosterone regulates lipid, carbohydrate and protein metabolism and modulates the functions of various cells and tissues, including muscle. In muscles, testosterone increases protein synthesis by stimulating androgen receptors and activating ways to increase insulin-like growth factor-1; in addition, it promotes the accretion of myonuclear cells and the involvement of satellite cells. Several studies have observed a cross-relationship between low testosterone levels and the development of weakness. [6,10]

To study pathologies of the neuromuscular system, mainly clinical research methods are used, among which electrophysiological methods, such as electromyography and electroneurography, which make it possible not only to evaluate the bioelectric activity of nerves and muscles, but also to study the rate of excitation through the nerve and through neuromuscular synapse [8].

In view of the foregoing, changes in the bioelectric activity of the neuromuscular system occurring in conditions of prolonged androgen deficiency were studied in more detail.

Materials and research methods. To achieve these goals, a complex of the sciatic nerve and gastrocnemius muscle and their synapse is used - a classic model of the neuromuscular system. In order to approximate the model to real conditions, studies were conducted in vivo [1].

The study was performed on 24 sexually mature white rats - Wistar males aged 5-6 months and weighing 180-260 g, which were divided into two groups: control (n = 10) and experimental (n = 14) ones. A model of a hypoandrogenic state was created by bilateral orchiectomy [1]. In the control group, only the opening of the scrotum membranes was performed, followed by suturing. Both groups of animals were kept under standard vivarium conditions (t 22 ± 2 °C, light / dark cycle - 12/12 h) on a standard diet for 120 days, after which the animals were involved in an acute experiment. For anesthesia, thiopental sodium was administered at a dose of 50 mg / kg weight. The prepared sciatic nerve was placed on bipolar irritating electrodes. The registration of the action potential (AP) from the calf muscle, as well as its direct irritation, was carried out using two needle electrodes. [1]

To record the responses received and their subsequent processing, standard equipment was used: an electronic stimulator, an amplifier, an analog-to-digital converter, and a personal computer.

Statistical processing of the study materials was performed with biometric analysis methods implemented in EXCEL-2003® and STATISTICA 6.1 (StatSoft Inc., Serial No. AGAR909E415822FA) licensed packages. The calculation of the percentages, arithmetic mean and the error of the mean (M ± m) were used. Probability was estimated using parametric statistics methods (Student's t test). Changes in indicators were considered probable at $p < 0.05$.

All experimental procedures were carried out in accordance with the European Council Directive of 24 November 1986 (86/609 / EEC).

Results and discussion. The main indicators of excitability were analyzed - the latent period (LP) of the appearance of the bioelectric response, the threshold of excitation, chronaxy, the amplitude and total duration of the action potential (AP) of the muscle during its indirect irritation, as well as the threshold and chronaxy of the calf muscle during its direct stimulation. The phenomenon of refractivity was studied by indirect double stimulation with an application interval of 1 to 20 ms, duration 0,3 ms, intensity 2 Th. Stimulation of the sciatic nerve with a series of 10 pulses and a frequency of 50 to 500 Hz was used to obtain data on changes in the processes of homosynaptic depression. [1]

The average value of the excitation threshold of the gastrocnemius muscle under indirect stimulation was 0.053 ± 0.004 mA (n = 10), in animals with experimental hypoandrogenemia it significantly ($p < 0.001$) increased to 433.96% (0.23 ± 0.005; n = 14). The size of chronaxy of the gastrocnemius muscle with indirect irritation was 45.5 ± 4.54 μs (n = 10), in animals with experimental hypoandrogenemia this indicator did not change significantly, only a tendency to decrease by 3.76% was observed (43.79 ± 1.23 μs; n = 14).

In order to study the excitability of the gastrocnemius muscle directly, the technique of its direct stimulation was applied. When examining the threshold, it was found that this indicator in the control group was 0.12 ± 0.001 mA (n = 10), and in the group of animals with experimental hypoandrogenemia it increased to 141.67% (in absolute values 0.17 ± 0.0075 mA $p < 0.01$, n = 10).

When studying chronaxy of the gastrocnemius muscle under direct stimulation conditions, it was found that this indicator was 70.6 ± 3.1 μs in the control group and decreased in the group of animals with experimental hypoandrogenemia to 60.48% (42.7 ± 1.48 μs, $p < 0.01$, n = 10).

As for the parameters of the evoked response, the duration of the latent period increased by 31.93% compared with the value in the control group, the amplitude of the AP increased by 65.44%, the duration of the AP increased by 101.99% ($p < 0.001$) (Table. 1).

Table 1

Parameters of the excitability of the gastrocnemius muscle with its indirect irritation, M ± m

AP parameters	control	Animals with EH
Latent period	1.19 ± 0.027 ms (n = 10)	1.57 ± 0.04 ms (n = 14) ***
Duration	3.51 ± 0.11 ms (n = 10)	7.09 ± 0.18 ms (n = 14) ***
Amplitude	20.95 ± 1.70 mV (n = 10)	34.66 ± 1.98 mV (n = 14) ***

Note: *** - confidence level $p < 0.001$

In response to indirect stimulation with double stimuli, starting from the interval of 3 ms, a trend was found to decrease the amplitude of AP in response to the second stimulus in the group of animals with experimental hypoandrogenemia. Changes value ranged from 27.08% to 11.6% ($p < 0.001$, $n = 14$) compared with the control group ($n = 10$). (Fig. 1).

Starting with a stimulation frequency of 50 Hz, in animals with orchiectomy, there was a clear significant tendency to a more pronounced in comparison with the control group index inhibition of the amplitude of the AP caused by the tenth stimulus. A rhythm transformation was observed with a decrease in the frequency of AP induced by 50% at stimulation frequencies above 200 Hz in the group with experimental hypoandrogenemia. In animals of the control group, rhythm transformation was observed at a stimulation frequency of more than 400 Hz (Fig. 2).

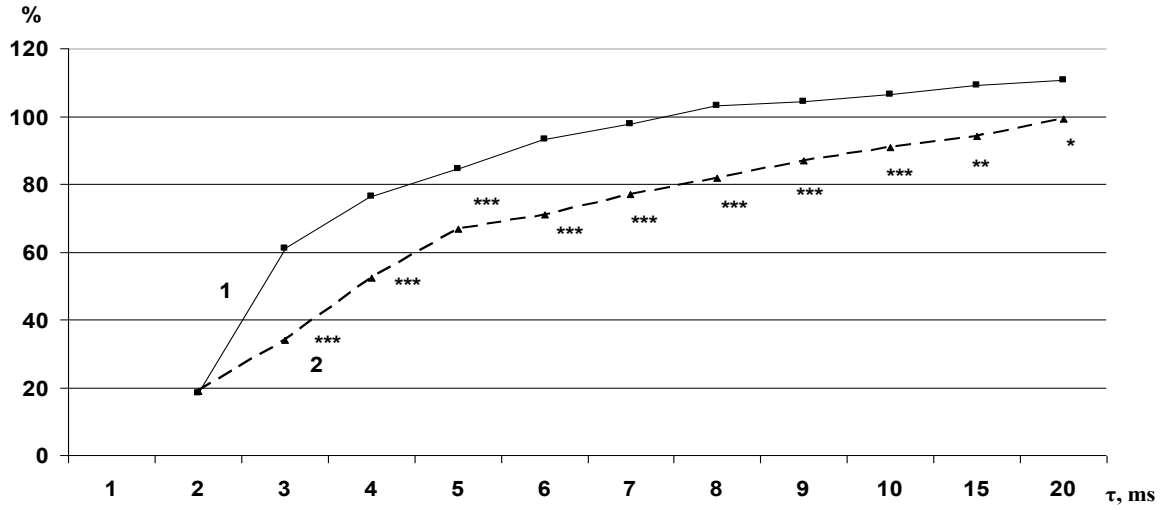

Figure 1 - Dynamics of changes in the amplitude of the action potential of the gastrocnemius muscle caused by a test stimulus in case of indirect irritation.
*The confidence level * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ in relation to the corresponding values of the control group. 1 - control animals; 2 - animals with experimental hypoandrogenemia.*

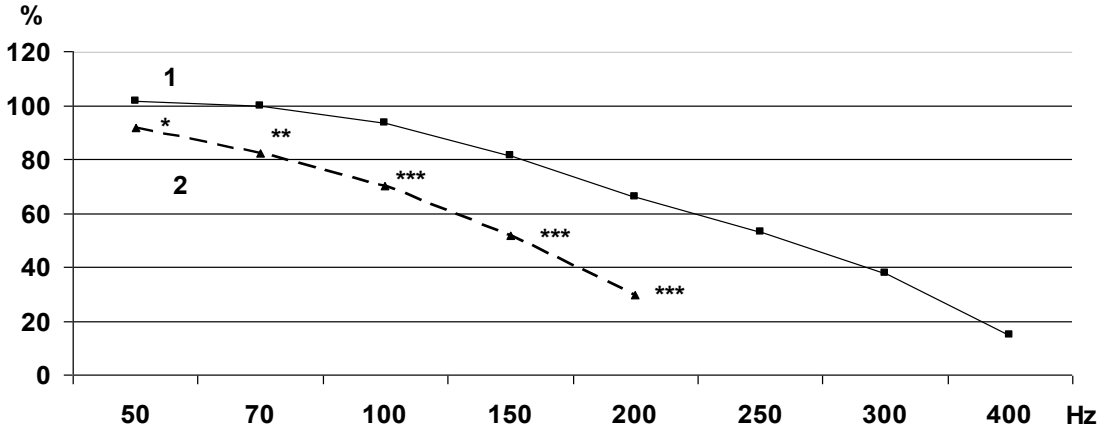

Figure 2 - The nature of the changes in the amplitude of the tenth potential of the gastrocnemius muscle during its indirect stimulation by a pack of 10 stimuli with a repetition rate of 50 to 500 Hz. 1 - control animals; 2 - animals with experimental hypoandrogenemia.

In the experiment, complete regeneration of striated muscle tissue in animals with normal androgen levels was accompanied by a short-term increase in the expression of SDF CHEMOKINE receptor 4 (CXCR4) and hepatocyte growth factor (HGF). The latter is probably an MSC activation signal, and CXCR4 initiates chemotaxis of the activated satellites towards the center of damage. Similarly, the expression of FST (follistatin) changed, which is associated with the control of filling during the regeneration process, and IGF-1, which is responsible for the differentiation of MSCs. [9]

Androgens also regulate the level of BDNF (neurotrophic factor of the brain) in target muscles. Testosterone treatment after castration restored the level of BDNF to the level of gonado-intact animals. [12]

It is likely that a violation of the processes of muscle tissue regeneration during natural renewal caused

by androgen deficiency caused a decrease in excitability in the calf muscle, which manifested itself as an increase in the threshold of excitation.

There is laboratory evidence that pharmacological testosterone treatment increases the level of mRNA encoding the synthesis of cholinacetyltransferase (CHAT) in the spinal cord motor neuron in adult male rats. An increase in the content of CHAT mRNA in motor neurons can potentially lead to an increase in the level of CHAT activity at the axon terminal, thereby increasing the presynaptic ability for the synthesis of acetylcholine [3].

It is possible that a lack of androgens causes opposite effects of impaired transmission of excitation through the neuromuscular synapse due to a decrease in the synthesis of acetylcholine. This may explain an increase in the phenomenon of homosynaptic depression and, as a result, a decrease in the lability of the neuromuscular apparatus as a whole (Fig. 2).

Testosterone has a neuroprotective effect in nerve fibers and cells. Castration leads to nerve degeneration, especially myelin, sheath degeneration. [2]

Treatment with androgens increases the ability of motor neurons to recover from regressive changes and restore both axons and dendrites, restoring normal neuromuscular function. [4]

So, testosterone deficiency can lead to various forms of nerve degeneration, which can ultimately lead even to anatomical changes [7]. This can explain the significant increase in the threshold of excitation in the gastrocnemius muscle with indirect stimulation, which was observed in the group of animals after orchiectomy.

There is evidence of a strong remyelinating effect of testosterone, which is derived from interaction with androgen receptors, in the curison model of sustained demyelination, where spontaneous recovery of myelin cannot be detected [7].

The violation of normal myelination of sciatic nerve fibers due to hypoandrogenemia, together with a distortion of their morphological structure, can lead to a decrease in the rate of excitation and, consequently, an increase in the duration of the latent period in animals from the experimental group (Table 1). Another reason for the increase in latency may be a decrease in the level of intracellular ionized calcium in the presynaptic terminals of motor neurons caused by a lack of androgens.

Androgens can interact with intracellular calcium regulators. A further effect of androgen exposure is a rapid change in $[Ca^{2+}]$ i [5]. And since modulation of calcium levels is a fairly quick reaction that occurs within a few seconds to several minutes, it is assumed that androgen must bind to some receptor on the cell surface to achieve this result [10].

Testosterone and its metabolites can interact with phospholipids in the membrane bilayer, changing the flexibility of the membrane and subsequently changing the function of sodium / potassium ATPase and calcium ATPase [5]. So, it was shown that molecules that interact with the lipid bilayer reduce the activity of both cation-activated adenosine-5-triphosphatases, such as sodium / potassium-adenosine-5-triphosphatase (Na / K-ATPase) and calcium-dependent adenosine- 5-triphosphatase (Ca^{2+} -ATPase) [5]. Testosterone, possibly, increases the activity of both Na / K-ATPase and Ca^{2+} -ATPase [6]. Acute testosterone therapy causes a dose-dependent increase in the hydrolytic ability of Ca^{2+} -ATPase isolated from the synaptosomal membranes of rat cerebral neurons [5].

So, a consequence of the lack of androgens can be a slowdown in the restoration of the transmembrane ion gradient due to a disruption in the functioning of ion pumps. The consequence of this is an increase in the duration of the refractoriness period, which was revealed in animals with a deficiency of male steroid hormones (Fig. 1).

It is known that K^+ channels in myocytes play a key role in the phase of repolarization of the action potential and its duration. Sex steroids can affect the function of K^+ channels by modulating gene expression and signaling pathways, which leads to sexual differences in electrophysiological and contractile functions. In addition, sex hormones directly bind to K^+ channels or auxiliary subunits to modulate the activity of blockers and activators of K^+ channels. So, testosterone increases repolarization current, ultra-fast current of K^+ channels, and expression of KV 1.5 protein (voltage-dependent K^+ channels), which leads to a shortening of the duration of the action potential of myocytes. [11] On the other hand, a lack of testosterone can lead to the opposite effect of lengthening the period of refractoriness, and therefore the total duration of AP and a decrease in lability (Table 1, Fig. 1).

The metabolite of dihydrotestosterone (DHT) 3α-diol can interact with the GABAA receptor and lead to an increase in intracellular calcium and, therefore, membrane potential [5]. Androgen deficiency can cause the opposite phenomenon - an increase in the transmembrane electric potential, which in turn can cause a compensatory increase in the amplitude of the induced response of the gastrocnemius muscle in castrated animals.

Conclusions. Thus, in the conditions of prolonged hypoandrogenemia, a marked decrease in excitability of the neuromuscular apparatus occurs, mainly due to nerve structures, taking into account a 4-fold increase in the threshold for the response of the gastrocnemius muscle during its indirect irritation. These changes were observed against the backdrop of a deterioration in temporal characteristics, namely: an increase in the duration of the latent period and the period of refractoriness caused by AP during indirect irritation of the calf muscle, and, as a consequence, a decrease in lability. The influence of the experimental state of neuromuscular connections was manifested in a decrease in the limiting frequency of transmission of excitation through synapses, which may indicate an increase in the phenomenon of homosynaptic depression. Unidirectional changes in the parameters of the excitability of the gastrocnemius muscle with indirect and direct irritation indicate the spread of the influence of low levels of male sex hormones in all parts of the neuromuscular complex.

However, the degree of influence of changes in the nerve fibers and central elements of the reflex arc on

the excitability of the neuromuscular apparatus and the temporal parameters of the induced responses remains not entirely clear. It is advisable to continue research on the impact of the hypoandrogenic state on the functioning of nerve fibers and motor neuron pool.

REFERENCES:

1. Rodinsky A.G., Tkachenko S.S., Mozgunov A.V. [Electrophysiological analysis of the excitability of the neuromuscular complex in experimental menopause]. Eksperymental`na ta klinichna fiziolohiya ta biokhimiya. 2014;67(3):7-13. Ukrainian. doi: https://www.ncbi.nlm.nih.gov/pubmed/19284114

2. Armagan A, Hatsushi K, Toselli P. The effects of testosterone deficiency on the structural integrity of the penile dorsal nerve in the rat. International Journal of Impotence Research. 2008;20:73-78. doi: https://doi.org/10.1038 / sj.ijir.3901614

3. Blanco CE, Zhan WZ, Fang YH, Sieck GC. Exogenous testosterone treatment decreases diaphragm neuromuscular transmission failure in male rats. J Appl Physiol. 2001;90:850-856. doi: https://doi.org/10.1152 / jappl.2001.90.3.850

4. Fargo KN, Foecking EM, Jones KJ. Neuroprotective actions of androgens on motoneurons. Front Neuroendocrinol. 2009;30(2):130-141. doi: https://doi.org/ 10.1016 / j.yfrne.2009.04.005

5. Foradori CD, Weiser MJ, Handa RJ. Non-genomic Actions of Androgens. Front Neuroendocrinol. 2008;29(2):169-181. doi: https://doi.org/ 10.1016 / j.yfrne.2007.10.005

6. Garibotto G, Picciotto D, Verzola D. Testosterone deficiency, frailty and muscle wasting in CKD: a converging paradigm? Nephrol Dial transplant. 2019;34(5):723-726. doi: https://doi.org/10.1093 / ndt / gfy295

7. Hussain R, Ghoumari AM, Bielecki B. The neural androgen receptor: a therapeutic target for myelin repair in chronic demyelination. Brain a journal of neurology. 2013;136:132-146. doi: https://doi.org/10.1093 / brain / aws284

8. Litchy WJ, Albers JW, Wolfe J, Bolton CF, Walsh N, Klein CJ, et set. Proficiency of nerve conduction using standard methods and reference values. Muscle Nerve. 2014;50(6):900-8. doi: https://doi.org/10.1002 / mus.24243

9. MacKrell JG, Yaden BC, Bullock H, Chen K, Shetler P, Bryant HU, Krishnan V. Molecular targets of androgen signaling that characterize skeletal muscle recovery and regeneration. Nuclear Receptor Signaling. 2015;13:1-19. doi: https://doi.org/ 10.1621 / nrs.13005

10. Narayanan R, Mohler ML, Bohl CE. Selective androgen receptor modulators in preclinical and clinical development. The Open Access Journal of the Nuclear Receptor Signaling Atlas. 2008;6:1-26. doi: https://doi.org/10.1621 / nrs.06010

11. Sakamoto K, Kurokawa J. Involvement of sex hormonal regulation of K + channels in electrophysiological and contractile functions of muscle tissues. J Pharmacol Sci. 2019;139(4):259-265. doi: https://doi.org/ 10.1016 / j.jphs.2019.02.009.

12. Verhovshek T, Rudolph LM, Sengelaub DR. BDNF and androgen interactions in spinal neuromuscular systems. Neuroscience. 2013;239:103-114. doi: https://doi.org/ 10.1016 / j.neuroscience.2012.10.028

EVALUATING THE EFFECTIVENESS OF PLETNEV DROPS NO. 1B, NO. 5 AND NO. 60 (DRUGS NO. 1B, NO. 5 AND NO. 60) IN THE TREATMENT OF PATIENTS WITH NONCARRYING OF PREGNANCY

Pletnev V.
Candidate of medical Sciences, doctoral student, senior researcher of Federal state budgetary institution "National medical research center of cardiology" of the Ministry of health of the Russian Federation

ОЦЕНКА ЭФФЕКТИВНОСТИ ПРИМЕНЕНИЯ КАПЕЛЬ ПЛЕТНЕВА №1В, №5 И №60 (ПРЕПАРАТОВ №1В, №5 И №60) ПРИ ЛЕЧЕНИИ ПАЦЕНТОК С НЕВЫНАШИВАНИЕМ БЕРЕМЕННОСТИ

Плетнев В.В.
Кандидат медицинских наук, докторант, старший научный сотрудник Федерального государственного бюджетного учреждения «Национальный медицинский исследовательский центр кардиологии» Министерства здравоохранения Российской Федерации

Abstract

Pletnev drops No. 1B, No. 5 and No. 60 are highly effective in the complex treatment of noncarrying of pregnancy for 3 months of reception according to the scheme: the first day – to take the drug No. 5 inside 5 drops, when dissolved in 50 ml of boiled water at room temperature 1 time a day in the morning before meals for 20 minutes; the second day – to take the drug No. 60 inside 5 drops, when dissolved in 50 ml of boiled water at room temperature 1 time a day before eating for 20 minutes; to take the drug No. 1B intravaginally 5 drops (0.25 ml), when dissolved in 70 ml of warm (37°-38°C) saline solution 1 time a day in the morning. With oral administration of drugs No. 5 and No. 60 and intravaginal administration of drug No. 1B, pregnancy occurred in 18 (90%) women. Normal delivery at 39-40 weeks was observed in 18 (100%) women. Drugs No. 1B, No. 5 and No. 60 do not cause