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ЕКСПЕРИМЕНТАЛЬНІ ДОСЛІДЖЕННЯ

THE IMPACT OF VITAMIN D AND/OR SAW PALMETTA BERRY EXTRACT ON THE SPERMOGRAMM AND SPERMATOZOA FUNCTIONING UNDER THE CONDITION OF EXPERIMENTAL BENIGN PROSTATIC HYPERPLASIA*

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The worsening of men's fertility which is observed during last years is mainly depended on the decreasing of number of sperm cells and/or the disturbance of their morphology. The tendency to the declining of number of spermatozoa in the ejaculate is detected in men worldwide. In spite the absence of strict correlation between fertility and normal morphological structure of sperm cells, the dead spermatozoa and sperms with changed morphology are significantly decreasing the onset of conceiving. The disturbance of the spermatozoa motility (asthenospermia) can be the sign of men's infertility, as well as can reveal the unsatisfactory condition and some risks for human health [1]. It is definitely that investigation of spermogram parameters is absolutely necessary in diagnosing of the causes of infertility. However, idiopathic abnormalities of spermogram indices are detected in 26 % of infertile men [2]. That because the treatment of infertility can be individual taking into account factors which have caused infertility, genetic component, environmental condition and comorbidities [3–5].

Benign prostatic hyperplasia (BPH) is one of the common pathology of men urogenital system. More than half of men after 40 years are suffering from this disease. It causes the disturbances of urination and intimate life problems [6]. The quantity and quality of seminal liquid are changed with this disease [7], which can lead to declining of men fertility. The significant number of side effects occurred during BHP medication encourages the develop-

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ment of new remedies for BHP prophylaxis and therapy.

The laboratory animals are used in the experimental medicine with the aim to design pathological conditions. The results of preclinical studies of drugs and conclusions which have been received about mechanisms of disease' development depend of the validity of this pathology modeling. The Sulpiride model is often used for investigation of prostate gland protective activity of medicines in rats. This model' essence lies in the prolonged sulpiride introduction which induces prolactin hypersecretion which in turn stimulates the proliferation of the Prostate gland (PG) glandular epithelium. The increased sensitivity of epithelial cells and 5-α-reductase activity determine development of BHP [8].

Vitamin D increasingly attracts the attention of scientific researchers. The data of current experimental and clinical researches are questionable as for its impact on the spermogram parameters which depend on level of vitamin D deficiency, its therapeutic or prophylactic dose, duration of treatment and comorbidity [9]. Thus, some authors point out that oral administration of vitamin D does not change the quantitative and qualitative parameters of spermogram in infertile men [10], whereas one randomized clinical trial has shown that the daily using of vitamin D in dose of 4000 IU by patients with asthenospermia during three months hasn't significantly altered the volume of ejaculate, the number and percentage of pathological form of spermatozoa, but has improved their motility [11]. The authors of systematic review with meta-analyses have confirmed vitamin D could improve motility and morphological structure, but didn't change the number of sperm cells in the ejaculate in infertile men [12]. The positive effect on the sperm quality, in particular, on spermatozoa motility can be explained by some factors linked with their functioning, that is direct nongenomic action of vitamin D on men' sperm [13].

Purpose. To determine the efficacy of cholecalciferol (vitamin D) administration, separately or together with Saw Palmetta berry extract, as for correction of spermogram in rat males under the condition of experimental sulpiride-induced benign prostatic hyperplasia.

MATERIALS AND METHODS

The investigations have been carried out in 12-month rat males with body mass of 330–380 g and according to the «National General Principles for Animal Researches Ethics» (Ukraine, 2001), which corresponds to the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasburg, 1986) and approved by the Bioethics Committee of the SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine».

BPH has been modeled by intramuscular injection of Sulpiride (Sulpiride-3H, solution for injection, 50 mg/kg) in dose of 40 mg/kg of animal body mass during 30 days (BPH group) in males. 0.9% Sodium chloride solution was daily introduced intramuscularly to animals of Control group (Control, n = 5). Starting from 31st day of the experiment the animals have received 4000 IU of cholecalciferol (vitamin D_3) during 21 days separately (group BPH + vit. D_3, n = 5) or together with reference medicine (group BPH + vit. D_3+Ref, n = 5).

Saw Palmetta berry extract (Serenoa repens) has been chosen to be the reference medicine (Ref) which is the active pharmaceutical ingredient of such widely used prostate gland protectors as Prostacare®, Prostamol® Uno, Prostaplant®. During experiment Saw Palmetta berry dried extract (produced by Ltd Research-an-Production Company «Vilarus», Ukraine) has been per orally introduced in dose of 35 mg/kg of body mass (BPH + Ref, n = 5). The solution of vitamin D_3 has been made with Apricot kernel oil and vitamin D_3 powder (China, lot CHG20062009, quality standard GB 9840-2017). Rat males were quick decapitated in two days after completion of introduction of investigated medicines, epididymis have been removed and suspension of sperm cells has been prepared. The efficiency of spermatogenesis was determined by the microscopic technic with the use of microscope «Biolam». The number of spermatozoa and the percentage of motile sperm cells (200 samples) have been calculated taking into account the shape of sperm cells' parts and the

presence of cytoplasmic drops. The concentration of morphologically normal cells C_N was calculated using equation [14]:

$$C_N = C \frac{100 - PF}{100}$$

wherein: C_N — the concentration of morphologically normal cells, 10^6/mL;
C — the concentration of spermatozoa from epididymis, 10^6/mL;
PF — percentage of pathological forms, %.

The functional capacity of spermatozoa has been evaluated by their osmotic resistance (in conventional units corresponded to concentration of Sodium Chloride solution under which the moving of cells is stopped), by the percentage of dead spermatozoa and duration of their moving.

The statistical estimation has been fulfilled using Shapiro-Wilk test to evaluate whether a data set is normally distributed. The probability of mean deviation has been determined according to Student`s t-test. Data were performed as arithmetic mean ($\bar{X}$) and standard error of the mean ($\pm S_{\bar{X}}$). The differences were considered to be significant at $P < 0.05$.

RESULTS AND THEIR DISCUSSION

The spermogram estimation has shown the percentage of motile spermatozoa was 77.8 ± 4.6 % in animals of control group. The concentration of sperm cells was 52 ± 2.5 10^6/mL and percentage of pathologically changed form was 4.8 ± 0.9% (Table 1).

The evaluation of spermogram of rats with sulpiride-induced BPH has detected all indices were significantly changed to compare to Control group. The motility of sperm cells and their general concentration have decreased by 45%, whereas the number of pathologically changed forms has increased by 67% ($p < 0.05$) (see Table 1, Fig. 1). This is why the integral index C_N has declined by 48%.

The percentage of dead spermatozoa was statistically higher by 4.4 times and duration of the motility of sperm cells has decreased by 30% in rats of BPH group (Table 2).

Spermogram evaluation has shown the percentage of motile spermatozoa has significantly increased by 1.7 times in rats with experimental BPH treated by vitamin D_3 (BPH + vit. D_3 group) comparing to BPH group. The same changes have been detected in rats with BPH after treatment by reference medicine (BPH + Ref group) and the percentage of motile sperm cells has increased by 1.9 times comparing to BPH group.

Concomitant use of vitamin D_3 and reference medicine in rats males with induced BPH (BPH + vit. D_3 + Ref group) has led to positive changes of spermogram indices. Thus, increasing of percentage of motile spermato-

Table 1

The parameters of spermogram of rat males with experimental BPH after treatment, ($\bar{X} \pm S_{\bar{X}}$), n = 5

Group	Index, spermatozoon			
	General concentration, 10^6/mL	C_N, 10^6/mL	Motile, %	Pathological forms, %
Control	52 ± 2.5	49.0 ± 2.8	77.8 ± 4.6	4.8 ± 0.9
BPH	28.4 ± 1.1[1]	25.33 ± 0.7[1]	43 ± 4.5[1]	14.6 ± 1.3[1]
BPH + Ref	41.2 ± 6.8	36.8 ± 6.6	79.8 ± 1.6[2]	11.4 ± 1.9
BPH + vit. D_3	35 ± 5.9[1]	30.7 ± 4.8[1]	74.4 ± 5.0[2]	11.8 ± 1.6[1]
BPH + vit. D_3 + Ref	43.6 ± 5.3[2]	39.5 ± 5.9[2]	72.4 ± 7.4[2]	11.2 ± 4.1

Notes:
[1] Statistically significant difference comparing to Control group ($p < 0.05$);
[2] Statistically significant difference comparing to BPH group ($p < 0.05$);
[3] Statistically significant difference comparing to BPH+Ref ($p < 0.05$);
C_N — concentration of morphologically normal spermatozoa;
BPH — benign prostatic hyperplasia.

Table 2

The indices of functional capacity of spermatozoa, ($\bar{X} \pm S_{\bar{x}}$), n = 5

Group	Index		
	Dead spermatozoa, %	Duration of spermatozoa motility, min	Osmotic resistance, CU
Control	3.2 ± 0.4	171.2 ± 30.3	2.4 ± 0.2
BPH	14.2 ± 2.0[1]	120.4 ± 12.6[1]	2.1 ± 0.1
BPH + Ref	8.8 ± 1.1 [1,2]	155.6 ± 28.6	2.3 ± 0.2
BPH + vit. D_3	7.2 ± 0.4 [1,2]	131.8 ± 15.2	2.1 ± 0.1
BPH + vit.D_3 + Ref	8.6 ± 0.7 [1,2]	151 ± 9.2	2.0 ± 0.1

Notes:
[1] Statistically significant difference comparing to Control group ($p < 0.05$);
[2] Statistically significant difference comparing to BPH group ($p < 0.05$);
[3] Statistically significant difference comparing to BPH+Ref ($p < 0.05$);
BPH — benign prostatic hyperplasia.

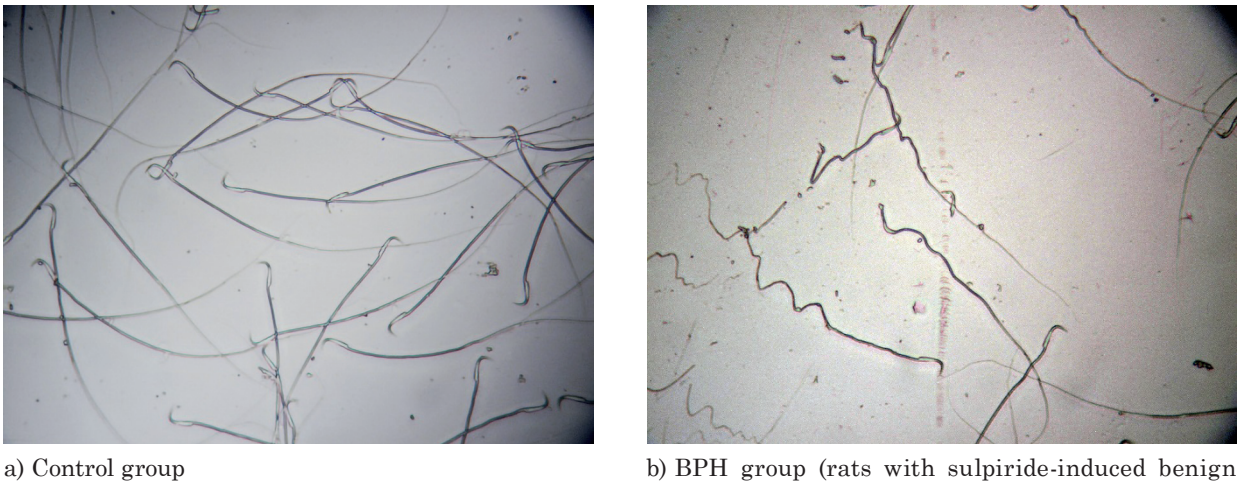
zoa by 1.7 times, the concentration of gametes — by 1.5 times and integral index C_N — by 1.6 times has been detected in males of BPH + vit. D_3 + Ref group comparing to parameters of spermogram of BPH group of rats.

During investigation of sperm functioning the duration of sperm motility and their osmotic resistance under condition of BPH and after administration of vitamin D separately or together with Ref haven't changed (see Table 2). However, the correction of BPH by researched medicines which used separately or together resulted in significant declining of percentage of dead spermatozoa almost by two times (see Table 2).

The spermatogenesis is considered not to be the ordinary cell differentiation. The result of this process is the forming of unique spermatozoon' components: acrosome, nucleus and tail (flagellum). It is well-known that normal mature sperms consist of «Head», «Mid (connecting) piece» and «Tail (flagellum)» the changes of shape of which define their pathology [15].

The analysis of sperms motility is important during estimation of fertility of men. There is Calcium-cationic sperm channel (CatSper) in the tail which is the activator of sperm cells motility [16].

It has been revealed that there is link between motility and extent of DNA fragmentation in males' sex cells: extent of fragmentation is declining with the growing of motile part of cells. At the same time the DNA fragmentation is growing with the number of non-motile spermatozoa is increasing [17]. The meaning link between qualitative and quantitative indices of



a) Control group

b) BPH group (rats with sulpiride-induced benign prostatic hyperplasia)

Fig. 1. Swabs of the rats epididymal spermatozoa, x400.

ejaculate is described in literature. It has been detected DNA fragmentation is increased in sperms due to declining of their concentration which is one of the indicators of inflammation of men reproductive system [18]. Our investigation has shown that the percentage of motile spermatozoa in Control group of animals (78%) and their high enough concentration suggested the ability to fertilization of these animals.

The «Sulpiride-induced model» which caused inflammation and sex hormones disbalance (insufficient function of PG disturbs the synthesis of testosterone in the testicles and promotes the increasing of the production of prolactin and estradiol) was used for BPH development in the experiment [17]. The prolonged use of Sulpiride stimulates the proliferation of glandular epithelium of PG which causes hyperprolactinemia. According to our data obtained the worsening of spermogram was detected under the condition of sulpiride-induced BPH. Scientific investigations in other species of animals have also confirmed that BPH has negative impacted the quality of sperm, the motility of spermatozoa has especially declined, which is linking with the growing of part of sperms producing nitrogen oxide [19].

The increasing of percentage of motile spermatozoa in males with BPH treated by cholecalciferol is closely related to researches of other authors pointed out positive effect of vitamin D metabolites on the motility and morphology of sperms [20]. Jueraitetibaike K. et al. (2019) have explained the mechanism of vitamin D influence on sperm motility. They came to conclusion that vitamin D can take part in the sperm' motile regulation in seminal plasma and its active form $1,25(OH)_2D$ can enhance the motility of gametes inducing ATP synthesis by cATP/PTK as well as by concentration of intracellular calcium ions to be increased [21].

The plant-based inhibitors of 5α-reductase are often administrated for BPH treatment. One of the most useful is Saw Palmetta Berry (*Serenoa repens*) extract [22]. It is known that *S. repens* extract inhibits the growing of PG tissues stimulated by dehydrotestosterone and estradiol [23]. The declining of prostate gland hyperplasia and improving of reproductive function are caused by the presence of unique complex of fatty acids including Lauric and Palmitic acids, Phytosterols and Triterpene saponins in the *S. repens* [24]. The androgen stimulating, anti-swelling, anti-inflammatory and vasoprotective effects of *S. repens* which were confirmed by clinical trials and experimental investigations, motivated us to use Saw Palmetta berry extract to be the reference medicine in our researches.

Some authors notice that *S. repens* extract along is practically useless to men with BPH and lower urinary tract symptoms, but in combination with other medicines can be potentially effective [25]. Our data obtained during experiment have confirmed that the use of Saw Palmetta berry extract in combination with vitamin D_3 has improved the spermogram parameters in rat males with BPH (BPH group + vit. D_3 + Ref).

CONCLUSIONS

1. Sulpiride-induced benign prostatic hyperplasia causes the disturbance of spermatogenesis in 12-month rat males which appears as a declining of number of motile gametes, decreasing of general sperm cells' concentration and increasing of pathological forms' number. The negative changes of spermatozoa functioning were manifested as a percentage of dead cells to be increased and as a declined duration of sperms moving.
2. The using of cholecalciferol for correction of benign prostatic hyperplasia causes the normalization of sperm cells' motility and decreasing of percentage of dead gametes in rats males with experimental pathology. The same effect was detected in males with modeled pathology treated by reference medicine.
3. Combined use of cholecalciferol and Saw Palmetta berry extract for correction of benign prostatic hyperplasia causes the normalization of spermatozoa concentration and their morphology, improving sperms motility and declining percentage of dead cells.

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THE IMPACT OF VITAMIN D AND/OR SAW PALMETTA BERRY EXTRACT ON THE SPERMOGRAMM AND SPERMATOZOA FUNCTIONING UNDER THE CONDITION OF EXPERIMENTAL BENIGN PROSTATIC HYPERPLASIA

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The tendency to the declining of number of spermatozoa in the ejaculate is detected in men worldwide. Benign prostatic hyperplasia (BPH) is one of the common pathology of men urogenital system. The infertility can be the consequence of this pathology. The significant number of side effects occurred during BHP medication encourages the development of new remedies for BHP prophylaxis and therapy. The laboratory animals are used in the experimental medicine with the aim to design pathological conditions. The Sulpiride model is often used for investigation of prostate gland protective activity of medicines in rats. This model' essence lies in the prolonged sulpiride introduction which induces BPH development. The experimental and clinical investigations demonstrate questionable data as for vitamin D influence on the spermogram parameters.

Purpose. To determine the efficacy of cholecalciferol (vitamin D) administration, separately or together with Saw Palmetta berry extract, as for correction of spermogram in rats males under the condition of experimental sulpiride-induced benign prostatic hyperplasia.

Materials and methods. BPH has been modeled by intramuscular introduction of Sulpiride in dose of 40 mg/kg to 12-month rat males with body mass of 330–380 g during 30 days. The correction of BPH was carried out by vitamin D_3 in dose of 4000 IU or saw Palmetta berry extract in dose of 35 mg/kg, separately or together, during 21 days after last injection of Sulpiride. Control group of animals have simultaneously received injection of Sulpiride and 0,9% solution of Sodium Chloride in dose of 0.5 mL. The spermatogenesis (sperms concentration, the percentage of motile and dead cells) and functional capacity of gametes have been evaluated in the suspension of spermatozoa obtained from epididymides of decapitated males. The probability of mean deviation has been determined according to t-Student test. Data were performed as arithmetic mean ($\bar{X}$) and standard error of the mean ($\pm S_{\bar{X}}$). The differences were considered to be significant at $P < 0.05$.

Results. It has been determined that Sulpiride-induced BPH has caused statistically significant changes of all spermogram' parameters which appears in the declined concentration and motility of gametes, in the increased number of pathological and dead forms of sperms comparing to Control group.

The correction of spermatogenesis disturbances by cholecalciferol or reference medicine has led to statistically significant increase in motile sperms percentage almost by two times and to the decrease in number of dead cells almost by two times in rats with BPH comparing to nontreated BPH group. Simultaneous use of vitamin D_3 with reference medicine has caused the increasing by 1.7 times of sperms motility, gametes concentration by 1.5 times, the concentration of morphologically normal cells by 1.6 times and decreasing of number of dead cells almost two times in rats with experimental pathology comparing to BPH group.

Conclusions. Sulpiride-induced benign prostatic hyperplasia causes the disturbance of spermatogenesis and functional capacity of spermatozoa in 12-month rat males. The correction of this pathology by cholecalciferol has improved sperm cells motility and has declined the percentage of dead cells. The same effect has been observed after administration of Saw Palmetta Berry extract. Simultaneous administration of these medicines has increased spermatozoa concentration, the number of morphologically normal gametes, sperms motility and has declined the percentage of dead sperm cells in rats with experimental benign prostatic hyperplasia.

Keywords: vitamin D_3, hyperplasia, prostatic gland, spermogram, cholecalciferol.

ВПЛИВ ВІТАМІНУ D ТА/АБО ЕКСТРАКТУ ПЛОДІВ ПАЛЬМИ САБАЛЬ НА СПЕРМОГРАМУ ТА ФУНКЦІОНАЛЬНИЙ СТАН СПЕРМАТОЗОЇДІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ДОБРОЯКІСНОЇ ГІПЕРПЛАЗІЇ ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ

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У чоловіків всього світу спостерігається тенденція до зменшення кількості та рухливості статевих клітин в еякуляті, що веде до погіршення чоловічої фертильності. Одним із розповсюджених захворювань сечостатевої системи чоловіків є доброякісна гіперплазія передміхурової залози (ДГПЗ). Велика кількість побічних ефектів, яка виникає при лікуванні ДГПЗ медикаментозними препаратами, спонукає до пошуку нових засобів профілактики та терапії цього захворювання.

Метою роботи було визначення ефективності застосування холекальциферолу (вітаміну D_3) окремо або сумісно з екстрактом плодів пальми Сабаль, щодо спермограми самців щурів при експериментальній сульпірид-індукованої доброякісній гіперплазії передміхурової залози.

Матеріали та методи. У 12-міс. самців щурів популяції Вістар масою 330–380 г моделювали ДГПЗ веденням в/м сульпіриду в дозі 40 мг/кг маси тіла протягом 30 діб. Після останньої ін'єкції сульпіриду протягом 21 доби проводили корекцію вітаміном D_3 у дозі 4000 МО або екстрактом плодів пальми Сабаль в дозі 35 мг/кг м.т. введенням *per os* окремо або сумісно. Контрольній групі під час ін'єкцій сульпіриду вводили 0,9% розчин хлориду натрію в дозі 0,5 мл. У епідидимальній суспензії вивчали стан сперматогенезу та оцінювали функціональну повноцінність гамет. Статистичну значимість відмінностей між групами визначали з використанням t-критерію Стьюдента. Розбіжності вважалися значущими при $p < 0,05$.

Результати. Показано, що сульпірид-індукована ДГПЗ призводить до статистично значущого зниження рухливості та концентрації сперматозоїдів, підвищення відсотку патологічних форм та мертвих гамет відносно групи Контроль. Корекція розладів сперматогенезу у щурів з ДГПЗ холекальциферолом або референтним препаратом призводила до статистично значущого підвищення відсотку рухливих сперматозоїдів майже в два рази, а також зниження майже в два рази відсотку мертвих клітин проти групи ДГПЗ без терапії. Сумісне введення віт. D_3 з референтним препаратом самцям з індукованою ДГПЗ призводило до збільшення в 1,7 рази рухливості сперматозоїдів, в 1,5 рази концентрації гамет та в 1,6 рази концентрації морфологічно нормальних клітин, та спостерігалось зниження майже в два рази відсотку мертвих гамет проти показників в групі ДГПЗ.

Висновки. У самців щурів 12-місячного віку при сульпірид-індукованій гіперплазії передміхурової залози виникають порушення процесу сперматогенезу та функціональної повноцінності сперматозоїдів. Корекція такої патології холекальциферолом призводить до нормалізації рухливості сперматозоїдів та зниження відсотку мертвих гамет. Аналогічна дія спостерігається при застосуванні екстракту плодів пальми Сабаль. Сумісне введення самцям із гіперплазією зазначених препаратів призводить до нормалізації концентрації сперматозоїдів, морфологічно нормальних гамет, рухливості сперміїв та зменшує відсоток мертвих статевих клітин.

Ключові слова: вітамін D_3, гіперплазія, передміхурова залоза, спермограма, холекальциферол.