EXPERIMENTAL WORKS

UDC 618.3-06:616.12-008.331.1]-036.1:577.161.2-022.252 **doi:** https://doi.org/10.15407/ubj97.04.034

EXPERIMENTAL PREECLAMPSIA DEVELOPMENT DEPENDS ON VITAMIN D_3 STATUS IN FEMALE WISTAR RATS

I. V. POLADYCH[1]✉, I. O. SHYMANSKYI[2], M. M. VELIKY[2], D. O. GOVSIEIEV[1]

[1]Department of Obstetrics and Gynecology No 1, Bogomolets National Medical University, Kyiv, Ukraine;
[2]Department of Biochemistry of Vitamins and Coenzymes, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
✉e-mail: iren.poladich@gmail.com

Received: 08 May 2025; **Revised:** 24 August 2025; **Accepted:** 12 September 2025

Deficiency of vitamin D_3 during pregnancy is a widespread challenge associated with increased risk of complications, particularly preeclampsia (PE), a serious condition characterized by hypertension with proteinuria. This research aimed to study the experimental preeclampsia rates in pregnant rats depending on the vitamin D_3 supply. Eight-week-old female Wistar rats were divided into three experimental groups: control; vitamin D_3-deficient for 60 days before mating; vitamin D_3-deficient with oral vitamin D_3 supplement (1000 IU/kg b.w.t) two weeks before mating. Subgroups with and without PE induction were analyzed. PE was induced by administration of Nω-nitro-L-arginine methyl ester (L-NAME). The blood level of vitamin D_3 was measured using a 25-Hydroxyvitamin D_3 ELISA kit. Proteinuria was assessed using semi-quantitative urine test strips "Prototest". The highest blood pressure and proteinuria levels were recorded in animals with combined vitamin D_3 deficiency and induced preeclampsia. Administration of vitamin D_3 contributed to normalization of hemodynamic parameters and kidney function, indicating the importance of an adequate vitamin D_3 status for pregnancy health and PE prevention.

K e y w o r d s: vitamin D_3 deficiency, L-NAME, preeclampsia, blood pressure, proteinuria level.

Vitamin D_3 (cholecalciferol) is currently recognized not only as a critical regulator of calcium-phosphate homeostasis and bone remodeling but also as a pleiotropic bioactive compound. It exerts significant effects through the vitamin D_3 auto-/paracrine system on cellular proliferation and differentiation, and participates in neurogenesis, angiogenesis, immune responses, and cell survival or apoptosis [1]. The hormonally active metabolite of vitamin D_3 – calcitriol ($1\alpha,25(OH)_2D_3$) – acts in cells via both non-genomic and genomic mechanisms, similar to other steroid hormones. These mechanisms are primarily mediated by the vitamin D_3 receptor (VDR) [2]. In target cells, the interaction of $1\alpha,25(OH)_2D_3$ with VDR initiates a complex cascade of molecular events culminating in the modulation of transcription factors activity that influences the expression of target genes [2-4].

Vitamin D_3 deficiency is a significant global health concern affecting various populations, including pregnant women [5-7]. Studies have shown that the prevalence of vitamin D_3 insufficiency among expectant mother's ranges from 28 to 96%, depending on geographic location, climate, dietary habits, and lifestyle [8]. On average, more than 65% of pregnant women have suboptimal vitamin D_3 levels, predisposing them to pregnancy-related complications [9].

Despite geographic differences in the prevalence of hypovitaminosis D_3 among pregnant women, its adverse effects on pregnancy outcomes are well documented, although the underlying mechanisms remain incompletely understood. Vitamin D_3 defi-

ciency has been associated with an increased risk of obstetric complications, including gestational hypertension, preeclampsia, preterm birth, fetal growth restriction, and higher rates of perinatal loss [10-12].

Among the most serious obstetric complications linked to vitamin D_3 deficiency is preeclampsia (PE) – a severe gestational disorder characterized by hypertension and proteinuria or signs of multiorgan dysfunction arising in the second half of pregnancy [13]. PE is a multifactorial pathological condition resulting from a combination of vascular dysfunction, impaired placental vascularization, chronic inflammation, and immune dysregulation.

To investigate the mechanisms of PE development and evaluate the effectiveness of therapeutic interventions, various experimental models using laboratory animals have been developed. These models aim to replicate the core pathophysiological features of PE, such as impaired placental blood flow, oxidative stress, low-grade chronic inflammation, and endothelial dysfunction. Among the commonly used experimental models of preeclampsia in rats are: surgical models, which involve uterine artery ligation or partial placental ischemia to simulate impaired uteroplacental blood flow characteristic of PE; genetic models, utilizing gene mutations or knockout animals to study the role of specific genes in PE pathogenesis; hormonal and immune-mediated models, based on administration of angiotensin II, TNF-α (tumor necrosis factor-alpha), or anti-VEGF (vascular endothelial growth factor) antibodies to induce pathology; pharmacological models, which rely on agents that provoke hypertension, vasoconstriction, and endothelial dysfunction [14].

In pharmacological approaches, the use of Nω-nitro-L-arginine methyl ester (L-NAME) – a nitric oxide synthase (NOS) inhibitor – has received particular attention [15]. L-NAME is widely used to induce PE in rodents due to its ability to inhibit nitric oxide (NO) production, a key modulator of vascular tone. When administered to pregnant rats, L-NAME induces hypertension, placental dysfunction, oxidative stress, and renal pathology, thus mimicking the clinical manifestations of PE in humans.

The main advantages of the L-NAME pharmacological model include its simplicity – administration can be oral, subcutaneous, or intraperitoneal – and it does not require complex or expensive equipment. The reproducibility of results makes this method a reliable and predictable model of PE, with characteristic biochemical and physiological alterations. Moreover, L-NAME is relatively inexpensive and widely available, making it ideal for experimental research. Importantly, its systemic action induces not only elevated blood pressure but also pathophysiological changes in the placenta, kidneys, and vasculature, thereby providing a comprehensive platform for investigating the cellular and molecular mechanisms underlying PE.

A relevant scientific challenge is to explore the mechanisms by which the pleiotropic effects of vitamin D_3 are realized during normal and complicated pregnancies. In particular, the relationship between alterations in vitamin D_3 status and vascular-endothelial dysfunction in PE remains insufficiently understood. In this context, the objective of our study was to assess the pathophysiological features of L-NAME-induced preeclampsia in rats with different levels of vitamin D_3 availability.

Materials and Methods

Animals and general experimental design. All experiments were conducted between November 2024 and February 2025 at the Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, in the Department of Vitamins and Coenzymes Biochemistry. A total of 37 eight-week-old female Wistar rats with an average body weight of 174 ± 12 g were used. Animals were divided into three groups (Table 1): group I (n = 10) – control females fed a standard vivarium diet without vitamin D_3 restrictions. Group II (n = 13) – females maintained on a rachitogenic diet devoid of vitamin D_3 for 60 days prior to mating to induce a vitamin D_3-deficient state. Group III (n = 14) – females also received a ricketogenic diet for 60 days, then switched to a standard laboratory diet with the ad-

Table 1. Experimental timeline for rat groups

Groups	Days 0–60	Days 61–74	Days 75–78	Days 79–100	GD5–GD15
I	Standard diet	Vehicle treatment	Mating	Pregnancy	L-NAME (±)
II	Rachitogenic diet	Vehicle treatment	Mating	Pregnancy	L-NAME (±)
III	Rachitogenic diet	Vit. D_3 treatment	Mating	Pregnancy	L-NAME (±)

dition of oral vitamin D_3 dissolved in sunflower oil in a volume of 0.2 ml administered via gavage (oil solution, 1000 IU/kg body weight, cholecalciferol; Sigma, USA) two weeks before mating. Animals were housed under standard vivarium conditions (22 ± 2°C, relative humidity 55–60%, 12-hour light/dark cycle), with ad libitum access to food and water. Group II animals were protected from direct sunlight exposure to prevent endogenous synthesis of vitamin D_3.

Ethical statement. All procedures were carried out in accordance with bioethical principles and international standards for the use of laboratory animals (EU Directive 2010/63/EU), including the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France; 1986) and the Guidelines for Bioethical Evaluation of Preclinical and Other Scientific Research Conducted on Animals (Kyiv, Ukraine; 2006).

Induction of vitamin D_3 deficiency. To induce vitamin D_3 deficiency, animals in groups II and III were fed a rachitogenic diet devoid of vitamin D_3 for 60 days before mating [16]. To confirm the development of vitamin D_3 deficiency and to monitor its status before mating and after the experimental endpoint, blood samples were collected from the tail vein and serum levels of 25-hydroxyvitamin D_3 ($25(OH)D_3$) were measured using a General 25-Hydroxyvitamin D_3 (HVD_3) ELISA kit (UNDL00047, Assay Genie). Sample concentrations were calculated using the Gain Data® (arigo's ELISA Calculator).

Mating and pregnancy monitoring. After confirming vitamin D_3 deficiency, the females were mated with fertile males at a male-to-female ratio of 2:1 (male : female). The presence of spermatozoa in vaginal smears the next morning was used to define gestational day 1 (GD1).

Induction of preeclampsia. To model L-NAME-induced preeclampsia (PE), each experimental group was subdivided into subgroups A and B. Subgroup A animals continued their respective dietary regimens. Rats in subgroup B received subcutaneous injections of L-NAME (Nω-nitro-L-arginine methyl ester, 100 mg/kg body weight) on GD5 and GD7. From GD5 to GD15, the drug was administered via drinking water at a dose of 40 mg/kg/day.

Blood pressure measurement. Systolic blood pressure was assessed using the CODA® Monitor (Kent Scientific, USA), a non-invasive tail-cuff system based on volume pressure recording (VPR). Measurements were taken twice: on GD1 (baseline) and GD15 (after 10 days of L-NAME treatment). During measurement, rats were placed in restrainers on a thermostatic platform (37–38°C) to ensure stable blood flow. The system recorded 3–5 readings within 5–7 min to minimize variability.

Assessment of proteinuria. Proteinuria was assessed using semi-quantitative urine test strips "PrototesT" (Norma, Ukraine). On GD15 of preeclampsia modeling, proteinuria was assessed by placing the rats in metabolic cages with free access to water to minimize stress. The indicator zone was moistened with a urine sample and assessed after 3 minutes by comparing color changes against a standard scale. The measurement range was 0–2.0 g/l.

Statistical analysis. Data were analyzed using conventional methods of variation statistics, with the calculation of the $M \pm m$. Statistical significance of differences between groups was assessed using one-way ANOVA followed by Bonferroni post hoc correction. Differences were considered significant at $P < 0.05$. Statistical processing was performed using Origin Lab 8.5 software.

Results and Discussion

The results of serum $25(OH)D_3$ levels – recognized as the primary marker of vitamin D_3 status and a precursor of its hormonally active form – demonstrated significant intergroup differences in pregnant Wistar rats (Table 2). In the control group, which received adequate vitamin D_3 intake, the highest serum levels of $25(OH)D_3$ were recorded: 49.8 ± 2.6 ng/ml in subgroup I A and 40.8 ± 3.7 ng/ml in subgroup IB, corresponding to optimal vitamin D_3 status by widely accepted standards [5, 6].

In pregnant rats from subgroup IIA, which were subjected to dietary vitamin D_3 restriction, $25(OH)D_3$ concentrations dropped to 17.0 ± 1.1 ng/ml, indicating a pronounced deficiency. Such a deficiency is typically associated with disturbances in calcium-phosphate metabolism and may increase the risk of gestational complications [7, 8]. In contrast, subgroup IIIA, which received cholecalciferol in an oil-based formulation to correct deficiency, showed a more than twofold increase in serum $25(OH)D_3$ levels (35.3 ± 2.2 ng/ml), demonstrating a high degree of therapeutic efficacy. The choice of lipid-based over water-soluble formulations was based on evidence from the literature, particularly studies by Durá-Travé T. et al. [17] and Wagner C.L. et al. [18], which emphasize the superior bioavailability of oil-based vitamin D_3.

Table 2. Serum 25(OH)D_3 levels in the studied rats (M ± m)

Indicator	Control (Group I), *n* = 10		D_3 Hypovitaminosis (Group II), *n* = 13		D_3 Hypovitaminosis + Vitamin D_3 (Group III), *n* = 14	
	IA (without PE), *n* = 5	IB (PE), *n* = 5	IIA (without PE), *n* = 7	IIB (PE), *n* = 6	IIIA (without PE), *n* = 7	IIIB (PE), *n* = 7
25(OH) D_3, ng/ml	49.8 ± 2.6	40.8 ± 3.7*	17.0 ± 1.1*^	–	35.3 ± 2.2*#	30.5 ± 1.2*#&^

Note. *Statistically significant difference compared to Group IA ($P < 0.05$); #statistically significant difference compared to Group IIA ($P < 0.05$); &statistically significant difference compared to Group IIIA ($P < 0.05$); ^statistically significant difference compared to Group IB ($P < 0.05$)

Importantly, 25(OH)D_3 levels were consistently higher in all subgroup A animals compared to their subgroup B counterparts, which were exposed to the nitric oxide synthase inhibitor L-NAME to model preeclampsia (e.g., 49.8 ± 2.6 ng/ml in IA vs. 40.8 ± 3.7 ng/ml in IB; 35.3 ± 2.2 ng/ml in IIIA vs. 30.5 ± 1.2 ng/ml in IIIB; $P < 0.05$). This may reflect both a direct impairment of cholecalciferol hydroxylation and an indirect effect of L-NAME-induced hypertension and impaired microcirculation on vitamin D metabolism, ultimately resulting in reduced synthesis of the vitamin D_3 prohormone. Rats in subgroup IIB, which experienced both vitamin D_3 deficiency and L-NAME-induced preeclampsia, did not survive. The high mortality rate in this subgroup may be attributable to inadequate physiological adaptation to L-NAME in the context of vitamin D_3 deficiency, late initiation and insufficient duration of vitamin D therapy, or the need for higher doses – issues that warrant further investigation.

Moreover, the lowest serum 25(OH)D_3 levels were observed in subgroup IIA compared to all other groups ($P < 0.05$), confirming the effectiveness of the model in inducing vitamin D_3 deficiency and validating the correction potential of cholecalciferol.

In summary, the obtained data indicate a significant influence of L-NAME on vitamin D_3 bioavailability as assessed by serum 25(OH)D_3. High-dose cholecalciferol supplementation in the setting of preeclampsia modeling may have practical implications for enhancing pharmacological strategies against this pregnancy complication, particularly when vitamin D_3 deficiency is present as a contributing pathophysiological factor.

In the next phase of the experiment, we assessed blood pressure dynamics in pregnant rats with different vitamin D_3 statuses and in the presence or absence of preeclampsia modeling. Blood pressure was measured on gestational day 1 (GD1) to determine baseline hemodynamic status, and repeated on GD15 – a critical period for fetoplacental development and for evaluating the preeclampsia model.

At the onset of pregnancy, both systolic (SBP) and diastolic (DBP) blood pressure values were within physiological norms across all groups (Table 3), with SBP ranging from 115.20 ± 4.48 to 122.60 ± 1.34 mmHg and DBP from 70.80 ± 3.26 to 78.70 ± 1.25 mmHg. This homogeneity in baseline values indicates the absence of systemic hypertension prior to experimental interventions, allowing for a valid assessment of each factor's contribution (vitamin D_3 deficiency, preeclampsia, vitamin D_3 supplementation) to hemodynamic changes.

By GD15, blood pressure varied significantly among groups, depending on vitamin D_3 status and preeclampsia modeling. In control rats (subgroup IA), blood pressure remained stable throughout pregnancy, confirming the protective role of adequate vitamin D_3 in maintaining normotension during gestation. In contrast, subgroup IIA animals, with vitamin D_3 deficiency, showed a moderate but statistically significant increase in SBP (132.00 ± 2.31 mmHg) and DBP (88.90 ± 2.04 mmHg) by GD15, even in the absence of additional stressors. This suggests that vitamin D_3 deficiency may act as an independent risk factor for gestational hypertension, potentially mediated by decreased bioavailability of endothelial NO, activation of the renin-angiotensin-aldosterone system (RAAS), and heightened sensitivity to vasoconstrictors [19, 20].

In subgroup III A, cholecalciferol-treated rats with prior D_3 deficiency maintained normotensive levels (SBP: 118.50 ± 4.27 mmHg; DBP: 77.00 ± 1.69 mmHg), further supporting the hypothesis that low vitamin D_3 status contributes directly to pregnancy-associated hypertension. The antihypertensive effect of vitamin D_3 may be mediated through vitamin D_3 receptor (VDR)-dependent

Table 3. Dynamics of blood pressure changes in pregnant rats at different gestational stages (M ± m)

Blood pressure, mmHg	Control (Group I), *n* = 10		D_3 hypovitaminosis (Group II), *n* = 13		D_3 hypovitaminosis + vitamin D_3 (Group III), *n* = 14	
	IA (without PE), *n* = 5	IB (PE), *n* = 5	IIA (without PE), *n* = 7	IIB (PE), *n* = 6	IIIA (without PE), *n* = 7	IIIB (PE), *n* = 7
Blood pressure on GD1						
SBP	120.60±0.87	112.60±3.57*	121.30±1.55	122.60±1.34	120.50±2.55	115.20±4.48
DBP	77.60±1.94	70,80±3.26*	78.40±1.34	78.70±1.25^	78.00±2.01^	74.80±4.23
Blood pressure on GD15						
SBP	110.20±4.56	163.80±2.76*	132.00±2.31*^	–	118.50±4,27#^	174.70±5.11*#&^
DBP	69.60±4.01	105.20±3.57*	88.90±2.04*^	–	77.00±1.69*#^	118.00±5.67*#&^

Note. *Statistically significant difference vs. Group IA ($P < 0.05$); #statistically significant difference vs. Group IIA ($P < 0.05$); &statistically significant difference vs. Group IIIA ($P < 0.05$); ^statistically significant difference vs. Group IB ($P < 0.05$)

anti-inflammatory pathways and improved vascular homeostasis. These effects may include downregulation of angiotensin II type 1 receptors (AT1R), improved NO bioavailability, and upregulation of pro-angiogenic factors such as VEGF and placental growth factor (PlGF) [21, 22]. This is consistent with other studies showing that vitamin D₃ reduces the expression of the antiangiogenic protein sFlt-1 (soluble fms-like tyrosine kinase-1), improves trophoblast invasion, and modulates RAAS function [23, 24].

In subgroup IIIB, which received L-NAME in addition to cholecalciferol therapy, blood pressure rose markedly (SBP: 174.70 ± 5.11 mmHg; DBP: 118.00 ± 5.67 mmHg). Notably, 100% mortality was observed in subgroup IIB (vitamin D_3 deficiency + L-NAME), likely due to acute hemodynamic instability. These findings suggest a synergistically detrimental effect of vitamin D_3 deficiency and preeclampsia-associated pathophysiological changes – particularly immune activation and endothelial dysfunction. Literature indicates that vitamin D_3 deficiency exacerbates immune dysregulation, inflammation, and oxidative stress, all of which contribute to angiogenic imbalance, a hallmark of preeclampsia pathogenesis [25, 26]. Additionally, vitamin D_3 deficiency impairs the synthesis of nitric oxide, a key vasodilator. This effect may amplify vascular tone when combined with the NO synthase inhibition induced by L-NAME [27].

It is noteworthy that preeclampsia modeling in control animals (subgroup IB) also led to a rise in blood pressure (SBP: 163.80 ± 2.76 mmHg; DBP: 105.20 ± 3.57 mmHg); however, these values were significantly lower than those observed in subgroup IIIB ($P < 0.05$). This suggests a partial attenuation of the adverse effects of preeclampsia under conditions of optimal vitamin D_3 status.

Our findings indicate that hypovitaminosis/deficiency of vitamin D_3 can be considered an independent risk factor for the development of gestational hypertension. Its insufficiency markedly exacerbates the hypertensive response in animals with L-NAME-induced preeclampsia. Vitamin D_3 supplementation resulted in a statistically significant reduction in blood pressure parameters, supporting its potential role as both a preventive and therapeutic agent in the management of preeclampsia.

Arterial hypertension may be closely linked to renal dysfunction and activation of the RAAS, while at the same time contributing to kidney injury and the development of renal failure. Proteinuria is a key marker of renal impairment and is widely used to assess the progression of nephropathies, including those associated with preeclampsia, which is often accompanied by glomerular filtration disturbances. The results of proteinuria assessment revealed marked intergroup differences, reflecting the functional status of the kidneys in pregnant rats depending on their vitamin D_3 status and hemodynamic alterations.

As shown in Figure, no proteinuria was detected in the urine of pregnant control females (subgroup IA), indicating the absence of structural kidney pathology in animals with sufficient serum

25(OH)D_3 levels. This underscores the importance of maintaining optimal vitamin D_3 status during pregnancy to preserve renal health [9]. In contrast, in control subgroup IB, 66.7% of the animals exhibited proteinuria at a level of 1.0 g/l, and 33.3% at 2.0 g/l – an indication of progressive nephropathy caused by L-NAME-induced preeclampsia, even in the presence of relatively adequate vitamin D_3 supply. This clearly highlights the multifactorial nature of preeclampsia, in which suboptimal vitamin D_3 status represents only one of many contributing pathophysiological mechanisms [10].

Given that 25(OH)D_3 concentrations were still significantly lower in preeclamptic control rats compared to healthy pregnant controls, it is plausible that higher-dose cholecalciferol supplementation could have provided more pronounced antihypertensive and nephroprotective effects in these animals.

In pregnant females of subgroup IIA, who did not receive correction for vitamin D_3 deficiency, proteinuria levels ranged from 0.15 to 0.5 g/l. Nearly half of the animals (42.9%) exhibited proteinuria levels of 0.3 g/l, indicative of moderate glomerular filtration impairment. This may represent the early stages of nephropathy, which could potentially progress to more severe forms in the context of vitamin D_3 deficiency [6]. As previously noted, the females in subgroup IIB, who were administered L-NAME, did not survive the experiment. This likely reflects significant pathophysiological disturbances in the organism, including possible renal involvement, which may have contributed to mortality.

In rats with partially corrected vitamin D_3 deficiency through cholecalciferol supplementation (subgroup IIIA), a moderate reduction in proteinuria levels was observed compared to the deficiency subgroup (IIA), correlating with reduced hypertension levels. The vast majority of animals in subgroup IIIA (85.7%) exhibited proteinuria around 0.15 g/l, supporting the potential nephroprotective effect of vitamin D_3. However, in the subgroup with induced preeclampsia against the background of vitamin D_3 correction (IIIB), proteinuria levels remained elevated: nearly 57% of the animals had urinary protein concentrations of 2.0 g/l. The degree of proteinuria in this subgroup exceeded that of the respective preeclamptic control animals (40%), aligning with higher blood pressure values and incomplete restoration of 25(OH)D_3 levels compared to the control. Thus, the observed intergroup differences demonstrate a clear relationship between the severity of arterial hypertension and proteinuria and serum 25(OH)D_3 levels under conditions of L-NAME-induced preeclampsia.

Overall, our results confirm that vitamin D_3 deficiency is associated with more severe renal injury in preeclampsia. This aligns with numerous clinical and experimental studies indicating that vitamin D_3 deficiency can disrupt glomerular filtration barrier function, increasing urinary protein excretion [28].

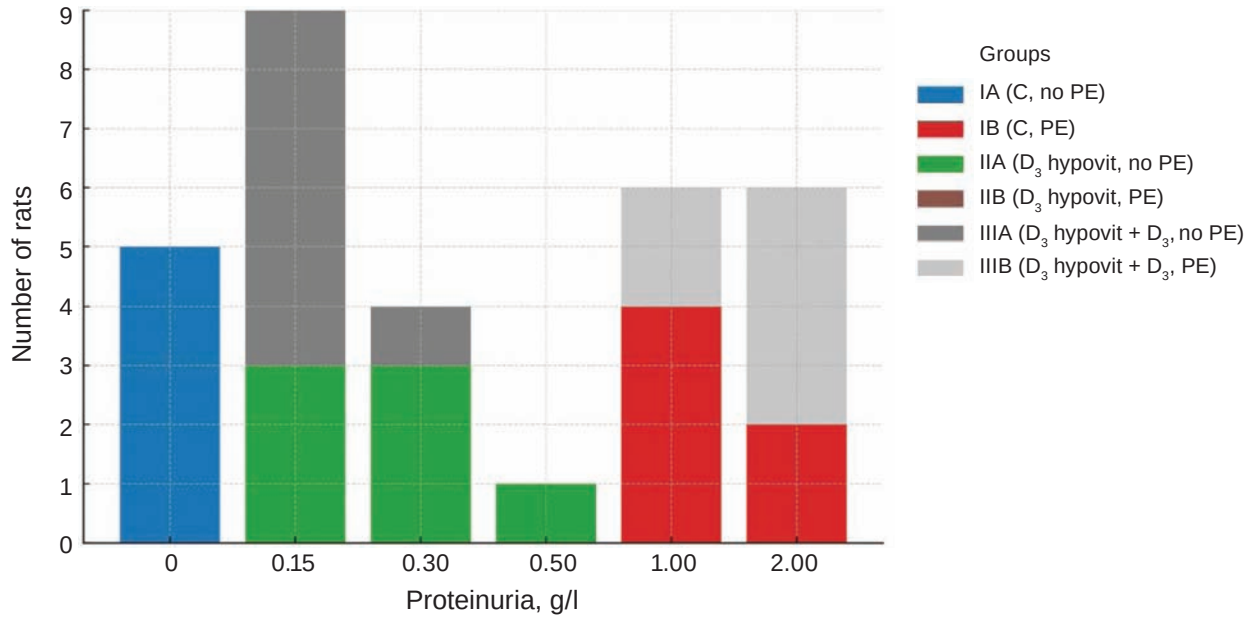

Figure. Distribution of female rats by level of proteinuria in the studied groups

Vitamin D_3 is believed to exert a protective effect on glomerular endothelial cells by reducing inflammation and oxidative stress – key pathogenic mechanisms in the development of preeclampsia. Specifically, low levels of vitamin D_3 have been shown to correlate with increased concentrations of inflammatory markers such as interleukin-6 and C-reactive protein, which may contribute to endothelial dysfunction and vascular tone dysregulation [29]. Vitamin D_3 insufficiency/deficiency has also been associated with reduced activity of antioxidant enzymes, exacerbating pro-oxidant processes and oxidative renal damage [30].

The data obtained underscore the need for routine monitoring of vitamin D_3 status in pregnant women and for its timely correction as a potential preventive measure against preeclampsia and its complications. Recent studies suggest the preventive effectiveness of vitamin D_3 supplementation in pregnant women at high risk for preeclampsia, with benefits for both maternal and fetal outcomes [31, 32].

Conclusions. The results indicate that vitamin D_3 deficiency in pregnant rats is associated with a significant increase in blood pressure and the development of proteinuria, implicating its involvement in the pathogenesis of preeclampsia. Administration of cholecalciferol contributed to normalization of hemodynamic parameters and kidney damage. The highest blood pressure values and proteinuria levels were recorded in animals with combined vitamin D_3 deficiency and induced preeclampsia, suggesting a synergistic effect of these factors. Therefore, the findings support the pathogenic role of vitamin D_3 deficiency in the development of gestational complications and highlight the potential of vitamin D_3 as a preventive and pathogenetic therapeutic agent in preeclampsia.

Practical implications. Routine monitoring of vitamin D_3 levels in pregnant women at high risk for preeclampsia is important for the prevention of vascular and renal complications. Correction of vitamin D_3 status may be an effective strategy to prevent preeclampsia and its complications, including hypertension and proteinuria, as demonstrated by the findings of this study and recent clinical research.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. This work was funded by the National Research Foundation of Ukraine (project No. 2023.03/0222).

ВІТАМІН-D_3 СТАТУС ЯК ВИЗНАЧАЛЬНИЙ ЧИННИК РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОЇ ПРЕЕКЛАМПСІЇ У САМИЦЬ ЩУРІВ ЛІНІЇ WISTAR

І. В. Поладич[1]✉, І. О. Шиманський[2], М. М. Великий[2], Д. О. Говсєєв[1]

[1]Національний медичний університет імені О. О. Богомольця, м. Київ, Україна;
[2]Інститут біохімії ім. О. В. Палладіна НАН України, Київ, Україна;
✉e-mail: iren.poladich@gmail.com

Дефіцит вітаміну D_3 під час вагітності є поширеною проблемою, що асоціюється з підвищеним ризиком ускладнень, зокрема прееклампсії (ПЕ) – серйозного стану, який характеризується артеріальною гіпертензією з протеїнурією. Метою цього дослідження було вивчення частоти розвитку експериментальної прееклампсії у вагітних щурів у залежності від забезпеченості вітаміном D_3. Самки щурів лінії Wistar віком 8 тижнів були розподілені на три експериментальні групи: контроль; дефіцит вітаміну D_3 протягом 60 днів до спаровування; дефіцит вітаміну D_3 з пероральною корекцією вітаміном D_3 (1000 МО/кг маси тіла) за два тижні до спаровування. Аналізувалися підгрупи з індукованою та неіндукованою ПЕ. Прееклампсію моделювали шляхом введення Nω-нітро-L-аргінін метилового естеру (L-NAME). Рівень вітаміну D_3 у крові визначали за допомогою набору ELISA для 25-гідроксивітаміну D_3. Протеїнурію оцінювали напівкількісними тест-смужками для сечі «Прототест». Найвищі показники артеріального тиску та рівня протеїнурії були зафіксовані у тварин із поєднаним дефіцитом вітаміну D_3 та індукованою прееклампсією. Введення вітаміну D_3 сприяло нормалізації гемодинамічних показників і функції нирок, що вказує на важливість адекватного вітамін-D_3 статусу для нормального перебігу вагітності та профілактики ПЕ.

Ключові слова: дефіцит вітаміну D_3, L-NAME, прееклампсія, артеріальний тиск, рівень протеїнурії.

References

1. Holick MF. The one-hundred-year anniversary of the discovery of the sunshine vitamin D_3: historical, personal experience and evidence-based perspectives. *Nutrients.* 2023; 15(3): 593.
2. Bikle DD. Vitamin D: newer concepts of its metabolism and function at the basic and clinical level. *J Endocr Soc.* 2020; 4(2): bvz038.
3. Carlberg C, Raczyk M, Zawrotna N. Vitamin D: A master example of nutrigenomics. Redox Biol. 2023; 62: 102695.
4. Carlberg C. Genomic signaling of vitamin D. *Steroids.* 2023; 198: 109271.
5. Grygorieva N, Tronko M, Kovalenko V, Komisarenko S, Tatarchuk T, Dedukh N, Veliky M, Strafun S, Komisarenko Y, Kalashnikov A, Orlenko V, Pankiv V, Shvets O, Gogunska I, Regeda S. Ukrainian consensus on diagnosis and management of vitamin D deficiency in adults. *Nutrients.* 2024; 16(2): 270.
6. Vestergaard AL, Christensen M, Andreasen MF, Larsen A, Bor P. Vitamin D in pregnancy (GRAVITD) - a randomised controlled trial identifying associations and mechanisms linking maternal Vitamin D deficiency to placental dysfunction and adverse pregnancy outcomes - study protocol. *BMC Pregnancy Childbirth*. 2023; 23(1): 177.
7. Suresh S, Patel E, Mueller A, Morgan J, Lewandowski WL, Verlohren S, von Dadelszen P, Magee LA, Rana S. The additive role of angiogenic markers for women with confirmed preeclampsia. *Am J Obstet Gynecol*. 2023; 228(5): 573.e1-573.e11.
8. Rouhani P, Mokhtari E, Lotfi K, Saneei P. The association between circulating 25-hydroxyvitamin D levels and preeclampsia: a systematic review and dose-response meta-analysis of epidemiologic studies with GRADE assessment. *Nutr Rev.* 2023; 81(10): 1267-1289.
9. Reddy M, Palmer K, Rolnik DL, Wallace EM, Mol BW, Da Silva Costa F. Role of placental, fetal and maternal cardiovascular markers in predicting adverse outcome in women with suspected or confirmed pre-eclampsia. *Ultrasound Obstet Gynecol*. 2022; 59(5): 596-605.
10. Schröder-Heurich B, von Hardenberg S, Brodowski L, Kipke B, Meyer N, Borns K, von Kaisenberg CS, Brinkmann H, Claus P, von Versen-Höynck F. Vitamin D improves endothelial barrier integrity and counteracts inflammatory effects on endothelial progenitor cells. *FASEB J.* 2019; 33(8): 9142-9153.
11. Ramdin S, Baijnath S, Naicker T, Govender N. The clinical value of rodent models in understanding preeclampsia development and progression. *Curr Hypertens Rep.* 2023; 25(6): 77-89.
12. Bakrania BA, George EM, Granger JP. Animal models of preeclampsia: investigating pathophysiology and therapeutic targets. *Am J Obstet Gynecol*. 2022; 226(2S): S973-S987.
13. Shu W, Li H, Gong H, Zhang M, Niu X, Ma Y, Zhang X, Cai W, Yang G, Wei M, Yang N, Li Y. Evaluation of blood vessel injury, oxidative stress and circulating inflammatory factors in an L-NAME-induced preeclampsia-like rat model. *Exp Ther Med*. 2018; 16(2): 585-594.
14. Nema J , Sundrani D , Joshi S . Prenatal vitamin D supplementation reduces blood pressure and improves placental angiogenesis in an animal model of preeclampsia. *Food Funct.* 2020; 11(12): 10413-10422.
15. Vitoratos N, Lambrinoudaki I, Rizos D, Armeni E, Alexandrou A, Creatsas G. Maternal circulating osteoprotegerin and soluble RANKL in pre-eclamptic women. *Eur J Obstet Gynecol Reprod Biol*. 2011; 154(2): 141-145.
16. Riasniy VM, Apukhovska LI , Veliky NN, Shymanskyy IO, Labudzynskyi DO, Komisarenko SV. Immunomodulatory effects of vitamin D_3 and bisphosphonates in nutritional osteoporosis in rats. *Ukr Biokhim Zhurn*. 2012; 84(2): 73-80. (In Ukrainian).
17. Durá-Travé T, Gallinas-Victoriano F. Pregnancy, Breastfeeding, and Vitamin D. *Int J Mol Sci*. 2023; 24(15): 11881.
18. Wagner CL, Hollis BW. The extraordinary metabolism of vitamin D. *Elife*. 2022; 11: e77539.
19. Bianconi V, Mannarino MR, Figorilli F, Cosentini E, Batori G, Marini E, Lombardini R, Gargaro M, Fallarino F, Scarponi AM, Sahebkar A, Pirro M. Prevalence of vitamin D deficiency and its prognostic impact on patients hospitalized with COVID-19. *Nutrition*. 2021; 91-92: 111408.
20. Verlohren S, Dröge LA. The diagnostic value of angiogenic and antiangiogenic factors in differential diagnosis of preeclampsia. *Am J Obstet Gynecol*. 2022; 226(2S): S1048-S1058.

21. Maugeri A, Barchitta M, Blanco I, Agodi A. Effects of vitamin D supplementation during pregnancy on birth size: a systematic review and meta-analysis of randomized controlled trials. *Nutrients.* 2019; 11(2): 442.
22. Abbasalizadeh S, Abam F, Mirghafourvand M, Abbasalizadeh F, Taghavi S, Hajizadeh K. Comparing levels of vitamin D, calcium and phosphorus in normotensive pregnant women and pregnant women with preeclampsia. *J Obstet Gynaecol.* 2020; 40(8): 1069-1073.
23. Benachi A, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, Senat MV, Haidar H, Jani J, Guizani M, Jouannic JM, Haguet MC, Winer N, Masson D, Courbebaisse M, Elie C, Souberbielle JC. Relationship between vitamin D status in pregnancy and the risk for preeclampsia: A nested case-control study. *Clin Nutr.* 2020; 39(2): 440-446.
24. Sparaco M, Bonavita S. Vitamin D Supplementation: Effect on Cytokine Profile in Multiple Sclerosis. *J Clin Med*. 2024; 13(3): 835.
25. Bi WG, Nuyt AM, Weiler H, Leduc L, Santamaria C, Wei SQ. Association between vitamin D supplementation during pregnancy and offspring growth, morbidity, and mortality: a ystematic review and meta-analysis. *JAMA Pediatr.* 2018; 172(7): 635-645.
26. Liu NQ, Ouyang Y, Bulut Y, Lagishetty V, Chan SY, Hollis BW, Wagner C, Equils O, Hewison M. Dietary vitamin D restriction in pregnant female mice is associated with maternal hypertension and altered placental and fetal development. *Endocrinology.* 2013; 154(7): 2270-2280.
27. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res.* 2019; 124(7): 1094-1112.
28. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. *Nutrients.* 2020; 12(2): 575.
29. Aouache R, Biquard L, Vaiman D, Miralles F. Oxidative Stress in Preeclampsia and Placental Diseases. *Int J Mol Sci*. 2018; 19(5): 1496.
30. Ma Y, Yang Y, Lv M, Zhang Y, He Q, Zhang Y, Su H, Deng X, Qian Y. $1,25(OH)_2D_3$ alleviates LPS-induced preeclampsia-like rats impairment in the protective effect by TLR4/NF-kB pathway. *Placenta*. 2022; 130: 34-41.
31. Poladych IV. Vitamin D in the genesis of preeclampsia: current understanding of the problem (literature review). *Bull Probl Biol Med.* 2024; 175(4): 113-122.
32. Lukyanova EM, Antipkin YuG, Omelchenko LI, Apukhovskaya LI. Vitamin D and its role in ensuring the health of children and pregnant women. Monograph. K. Iz-vo “Expert”. 2005. 230 p.