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ROLE OF VITAMIN D IN THE DEVELOPMENT OF FETAL LOSS SYNDROME. PRIMARY RESULTS OF A COHORT STUDY

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Abstract. Given the dynamics of the decline in the birth rate in Ukraine during wartime and the demographic crisis, the issue of studying the factors of reproductive losses and creating effective measures for their prevention is an extremely urgent task. The purpose of the work is to assess the role of vitamin D in the development of fetal loss syndrome. A cohort study was conducted with the participation of 83 women of reproductive age from 21 to 42 years (on average - 30.86 ± 5.02 years) with a history of fetal loss syndrome, who sought medical help from obstetric and gynecological hospitals in Vinnytsia during 2017-2022. The comparison group included 83 women with a normal course of pregnancy and childbirth. The level of vitamin D was assessed by the concentration of 25-OH vitamin D total (vitamin D2 and vitamin D3) in blood serum, which was determined by the enzyme-linked immunosorbent assay (ELISA). Statistical processing of the obtained data was carried out using the statistical package of information processing SPSS 21 (©SPSS Inc.). The results of the study showed that the level of 25(OH)D was statistically significantly lower in the group of women with fetal loss syndrome, in particular with detected hemostasis disorders, compared with women with a normal course of pregnancy and childbirth ($p < 0.05$). The average level of 25(OH)D in serum was 21.74 ± 9.37 ng/ml in women with fetal loss syndrome and 38.31 ± 5.96 ng/ml in patients of the comparison group. It was found that 77.1% of women with fetal loss syndrome had insufficiency (27.7%) or deficiency (49.4%) of vitamin D in serum. At the same time, in women of this category, the indicated vitamin D statuses were significantly more frequent than in women with a normal course of pregnancy and childbirth (OR 4.92; 95% CI [1.88-12.8] and OR 82.0; 95% CI [10.89-617.3], respectively). Therefore, the conducted studies have established a significant relationship between vitamin D deficiency and fetal loss syndrome, in particular in women with hemostasis disorders, which requires studying the effectiveness of its use to improve pregnancy outcomes.

Keywords: fetal loss syndrome, miscarriage, early miscarriages, pregnancy, risk factors, vitamin D, thrombophilia.

Introduction

According to various authors, the frequency of fetal loss syndrome ranges from 2% to 55% and includes two or more spontaneous abortions at 10 weeks of gestation or more, three or more spontaneous abortions at the preembryonic or early embryonic stage, when anatomical, genetic and hormonal causes are excluded.

causes of miscarriage, stillbirth and neonatal death due to premature birth or placental insufficiency [11, 17]. It is known that the risk of other serious obstetric and psychological complications, including preeclampsia, stillbirth, depression and post-traumatic stress disorder, is increased in women of this category [5]. Given the dynamics of the decline in the birth rate in Ukraine in the context of wartime and demographic crisis, the issue of studying the factors of reproductive losses and creating effective measures for their prevention is an extremely urgent task.

Women with fetal loss syndrome often have uncertainty about the cause and likelihood of recurrence [5]. In this regard, research has focused on examining factors related to genetics, age, antiphospholipid syndrome, uterine abnormalities, thrombophilia, hormonal or metabolic disorders, infection, sperm quality, and problems

lifestyle [11, 12]. These publications do not support definitive conclusions about the causes of fetal loss syndrome, as most studies of pregnancy loss have focused only on sporadic miscarriages.

Vitamin D deficiency (low serum levels of 25-hydroxyvitamin D – 25(OH)D) is now recognized as a serious global health problem. Pregnant women and those planning pregnancy are at increased risk of altered vitamin D status [6, 8]. Prevalence ranges from 5% to 90% depending on the country [2, 7].

Current research suggests that vitamin D deficiency is common in women with serious obstetric complications, including preeclampsia, gestational diabetes, and preterm labor [2, 3, 19]. According to a meta-analysis by JA Tamblyn et al. (2022), vitamin D deficiency and insufficiency are associated with an increased risk of spontaneous abortion [14]. However, it is currently uncertain whether preconceptional correction of vitamin D deficiency can reduce the risk of pregnancy loss in women in this category. A similar question applies to patients with fetal loss syndrome. This question determined the purpose of our study, the initial results of which are presented in this publication.

The aim of our work is to assess the role of vitamin D in the development of fetal loss syndrome.

Materials and methods

A cohort study was conducted with the participation of 83 women of fertile age from 21 to 42 years (average (M±y) – 30.86±5.02 years) with a history of fetal loss syndrome, who sought medical help from obstetric and gynecological hospitals in Vinnytsia during 2017-2022. These women were included in the main group.

The inclusion criteria for the study were:

- 1) >1 spontaneous abortion at 10 weeks of gestation or more (including non-developing pregnancy);
- 2) 3 or more spontaneous miscarriages at the preembryonic or early embryonic stage, when there are no anatomical, genetic and hormonal causes miscarriage;
- 3) stillbirth;
- 4) neonatal death due to premature birth, severe preeclampsia, or placental insufficiency.

The exclusion criteria for the study were:

- 1) chromosomal aberrations;
- 2) anatomical defects;
- 3) severe endocrine disorders;
- 4) male factor infertility in women with preembryonic losses and in vitro fertilization failures nition.

The comparison group consisted of 83 women with normal pregnancy and childbirth aged 20 to 44 years (average (M±y) – 33.11±6.14 years).

The patient groups were formed using the "case-control" method, therefore the analysis of demographic and anthropometric indicators, somatic and reproductive health did not reveal statistically significant differences between them.

During the analysis of medical documentation, reproductive function was studied: age of menarche, somatic, gynecological and obstetric anamnesis, results of the study of polymorphism of thrombophilia genes. Identification of genetically determined forms of thrombophilia was carried out by molecular genetic analysis in 3 stages: DNA isolation, amplification (by polymerase chain reaction), restriction.

Vitamin D levels were determined in the first trimester of pregnancy by the concentration of 25-OH vitamin D (vitamin D D2 and vitamin D3) in the blood serum using the enzyme-linked immunosorbent assay (ELISA) on a strip enzyme-linked immunosorbent assay analyzer "Humareader single" (Germany). Blood samples for research were taken from the cubital vein in the morning on an empty stomach.

Vitamin D status was assessed according to recommendations, according to which the reference level of 25(OH)D in serum should be >30 ng/ml, suboptimal level (vitamin D deficiency) – 20-29.9 ng/ml, deficiency – <19.9 ng/ml, in particular moderate deficiency – 10-19.9 ng/ml, severe deficiency – <10 ng/ml [1].

Statistical processing of the obtained data was carried out using the statistical package for medical and biological research ("SPSS", version 20, "IBM").

Data are presented as M±y (mean ± standard deviation). To find frequency differences, the y2 (Pearson) method was used, and the odds ratio (OR) was determined.

and 95% confidence interval (CI). Differences were considered statistically significant at p<0.05 (95% level of significance).

The research was carried out by the authors as part of their initiative work.

Results

The main clinical and demographic characteristics of women in the main group are presented in Table 1.

Table 1. Main clinical and demographic characteristics of the main group of women (n=83).

Indicator	Indicator value	
	M±y	abs. (%)
Age, years	30,86±5,02	-
Accommodation in the city	-	56 (67,5 %)
Married	-	74 (89,2 %)
Body weight, kg	65,61±15,63	-
Body mass index (BMI), kg/m2	23,69±5,31	-
Presence of comorbid somatic pathology	-	40 (48,2 %)

Note: the table shows the arithmetic mean values of the studied indicators (M) and standard square deviations (y).

The structure of reproductive losses in the main group is as follows: 34 women (41%) had spontaneous abortions at gestational age 10 and above, 12 (14.5%) had spontaneous late abortions (after 12 weeks of pregnancy), 26 (31.3%) and 8 (9.6%) had stillbirths in early and late pregnancy, respectively. 4 of the examined women (4.8%) had a history of antenatal fetal death, and 2 (2.4%) had early neonatal death due to premature birth (1 case) and severe preeclampsia (1 case).

The majority of the examined women in the main group with fetal loss syndrome, in particular 55 people (66.3%) had thrombophilia. The serum level of 25(OH)D

in the patients in the main group was on average 21.74±9.37 ng/ml, which was 1.8 times lower than in the women in the comparison group – 38.31±5.96 ng/ml (p<0.05) (Table 2).

Table 2. Differences between the average indicators of the level 25(OH) D in the blood serum of women in the main group and the comparison group.

Indicator	Main group (n=83)	Comparison group (n=83)
	M±y	M±y
25(OH)D, ng/ml	21,74±9,37*	38,31±5,96

Notes: the table shows the arithmetic mean values of the studied indicators (M) and standard square deviations (y); - the level of significance of the differences in indicators compared to the other group p<0.05.

When detailing the vitamin D status in the analyzed women, it was found that 95 out of 166 patients (57.2%) had an optimal level of 25(OH)D in the blood serum, suboptimal level (20-29.9 ng/ml) – 29 (17.5%) and vitamin D deficiency with serum level <19.9 ng/ml – 42 women (25.3%). At the same time, a significantly larger number of women in the main group had a lack and deficiency of vitamin D – 23 (27.7%) and 41 (49.4%) respectively, against 6 (7.2%) and 1 (1.2%) patients in the comparison group – OR 4.92; 95% CI [1.88-12.8], p<0.001 and OR 82.0; 95% CI [10.89-617.3], p<0.001, respectively (Table 3).

Table 3. Serum vitamin D status of women in the main group and comparison group.

25(OH)D, ng/ml	Main group (n=83)	Comparison group (n=83)	SS [95% CI]
	abs. (%)	abs. (%)	
>30	19 (22,9 %)	76 (91,6 %)*	
20-29,9	23 (27,7 %)	6 (7,2 %)*	4,92 [1,88-12,8]
<20	41 (49,4 %)	1 (1,2 %)*	82,0 [10,89-617,3]

Notes: the table shows the arithmetic mean values of the studied indicators (M) and standard square deviations (y); - the level of significance of the differences in indicators compared to the other group p<0.001.

Discussion

During pregnancy, a woman's body undergoes significant physiological changes, including the need for and metabolism of vitamin D, so vitamin D deficiency during gestation is a common problem both in Ukraine and throughout the world [2, 7].

According to the results of the study, 77.1% of women with fetal loss syndrome had insufficiency (27.7%) or deficiency (49.4%) of vitamin D in their blood serum.

There are several meta-analyses that have examined the relationship between vitamin D status during pregnancy and maternal and neonatal outcomes [9, 15], including the effects of vitamin D supplementation during pregnancy on these outcomes [10, 16]. However, the association of vitamin D insufficiency or deficiency with fetal loss syndrome,

and its individual variants, is poorly studied. There is also currently insufficient evidence to accurately assess whether preconceptional correction of vitamin D deficiency reduces the risk of pregnancy loss in women in this category [14].

Our results suggest that vitamin D insufficiency and deficiency are more common in women with fetal loss syndrome than in women with normal pregnancy and labor (OR 4.92; 95% CI [1.88-12.8] and OR 82.0; 95% CI [10.89-617.3], respectively). The results of the study add to the current evidence that women with vitamin D deficiency have an increased risk of miscarriage in addition to other serious obstetric complications and pregnancy outcomes [3, 4]. These data support the important role of vitamin D before conception and/or in early pregnancy, as the placenta is the major tissue for the accumulation of both 25(OH)D and active 1,25-dihydroxyvitamin D, 1,25(OH)2D [13], which influences trophoblast invasion, placental spiral artery remodeling, and immune cell function [18]. Therefore, practitioners should consider that low serum 25(OH)D levels in women are likely to be involved in the pathophysiology of pregnancy loss through concomitant reductions in placental 1,25(OH)2D and associated placental dysregulation.

Conclusions and prospects for further developments

1. 77.1% of women with fetal loss syndrome had serum vitamin D insufficiency (27.7%) or deficiency (49.4%).
 2. It was found that vitamin D insufficiency and deficiency are more common in women with fetal loss syndrome than in women with normal pregnancy and childbirth (OR 4.92; 95% CI [1.88-12.8], p<0.001 and OR 82.0; 95% CI [10.89-617.3], p<0.001, respectively).
- Given that our study confirms a significant association between vitamin D deficiency and fetal loss syndrome, particularly in women with hemostasis disorders, the prospect of further research is to study the effectiveness of using vitamin D to improve pregnancy outcomes.

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THE ROLE OF VITAMIN D IN THE PATHOGENESIS OF PREGNANCY LOSS SYNDROME: PRIMARY RESULTS OF A COHORT STUDY

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Annotation. Considering the dynamics of declining birth rates in Ukraine during wartime and the demographic crisis, the investigation of factors contributing to reproductive losses and the development of effective preventive measures is an extremely relevant task. Objective – to assess the contribution of vitamin D to the development of pregnancy loss syndrome. A cohort study was conducted involving 83 women of reproductive age (21 to 42 years, mean age 30.86±5.02 years) with a history of pregnancy loss syndrome who sought medical care at obstetric and gynecological hospitals in Vinnytsia between 2017 and 2022. The comparison group included 83 women with normal pregnancy and childbirth outcomes. Vitamin D levels were assessed by measuring the total 25-hydroxyvitamin D (vitamin D2 and vitamin D3) concentration in serum using the enzyme-linked immunosorbent assay (ELISA) method. Statistical processing of the obtained data was performed using the SPSS 21 statistical software package (©SPSS Inc.). The results of our study show that the level of 25(OH)D was statistically significantly lower in the group of women with pregnancy loss syndrome, including those with detected hemostasis disorders, compared to women with normal pregnancy and childbirth outcomes ($p < 0.05$). The mean serum 25(OH)D level was 21.74±9.37 ng/mL in women with pregnancy loss syndrome and 38.31±5.96 ng/mL in the comparison group. It was found that 77.1 % of women with pregnancy loss syndrome had either vitamin D insufficiency (27.7 %) or deficiency (49.4 %) in their serum. Moreover, in this category of women, these vitamin D statuses were significantly more frequent than in women with normal pregnancy and childbirth outcomes (OR 4.92; 95 % CI [1.88-12.8] and OR 82.0; 95 % CI [10.89-617.3], respectively). So based on the conducted studies, a significant relationship between vitamin D deficiency and fetal loss syndrome has been established, including in women with hemostasis disorders, which requires studying the effectiveness of its use to improve pregnancy outcomes.

Keywords: pregnancy loss syndrome, miscarriage, early pregnancy loss, pregnancy, risk factors, vitamin D, thrombophilia.