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ORIGINAL ARTICLE

Study on the clinical value of Vitamin D in recurrent spontaneous abortion

Revisiting the Importance of Vitamin D

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Abstract

Objective: This study explores the possible pathogenesis of recurrent spontaneous abortion (RSA) caused by vitamin D (VD), provides evidence-based bases for prevention and treatment of RSA, improves female reproductive health.

Methods: This study randomly selected 305 patients without spontaneous abortion (SA0), 216 patients with a spontaneous abortion (SA1) and 200 patients with RSA from 1421 women of childbearing age who visited the RSA specialty clinic of Hangzhou First People's Hospital from January 2021 to June 2023 to conduct a prospective clinical study. Then, we collected the data of clinical diagnosis and treatment, conducted intervention and follow-up, and finally executed statistical analysis.

Results: (1) RSA patients were significantly older than the other two groups. (2) The rates of VD deficiency in SA1 and RSA patients were significantly higher than those in SA0. (3) When BMI < 20 or > 24 kg/m^2, there were abnormal increase in VD and increased number of spontaneous abortions. (4) The bilateral S/D of the VD-sufficient, VD-insufficient and VD-deficient groups gradually increased with statistical significance ($p \leq .018$). (5) Among the 65 cases undergoing embryo chromosome examinations, chromosomal abnormalities accounted for 55.38% and 69.05% in RSA patients. (6) Among 186 patients with abnormal ACA, there was a certain negative correlation between ACA and VD, which was stronger among RSA patients. Moreover, ACA significantly decreased ($p < .001$) after effectively supplementing VD, and the miscarriage rate of re-pregnancy also decreased.

Conclusion: The rate of VD deficiency is higher in RSA patients. VD deficiency may be related to the age of women of childbearing age and too low or high BMI, and may cause abnormal plasma antiphospholipid antibodies, increased uterine artery resistance and abnormal chromosomal division during fertilization, leading to spontaneous abortion and even RSA. The improvement of VD deficiency may reduce the risk of RSA occurrence.

Wenqi Du and Chao Ye contributed equally to this work and both acted as first authors.

KEYWORDS
age, anticardiolipin antibody, BMI, embryonic chromosome, recurrent spontaneous abortion, uterine artery resistance, vitamin D

1 | INTRODUCTION

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive spontaneous abortions.[1] In recent years, the incidence rate of RSA has risen gradually, affecting 2—4% of women of childbearing age.[2] The risk of RSA, which is more than 80% after three times, increases by 10% with an increase in the number of abortions.[3,4] However, there are still 50% unknown causes of RSA.[5] Therefore, RSA brings significant challenges to clinical diagnosis and treatment, places economic and mental burdens on many families and makes it an important issue that urgently needs to be addressed in the field of reproductive health.

A recent study[6] has shown that among the maternal factors leading to RSA, pre-thrombotic state (PTS) is an important cause of spontaneous abortion, and it is not an isolated pathological reaction process. In addition to genetic PTS caused by gene mutations related to coagulation anticoagulation, it is more secondary to acquired PTS in various diseases such as immunometabolic diseases. Moreover, many researches have shown that abnormal expression of the receptor molecules of human leukocyte antigen at maternal-fetal interface may induce systemic inflammation, causing preeclampsia, spontaneous abortion, and even RSA[7,8]; hyperinsulinemia, insulin resistance, hyperglycemia and endothelial damage in polycystic ovary patients can induce blood hypercoagulability,[9] leading to RSA. Vitamin D (VD), a steroid derivative, plays an irreplaceable role in reproductive immunity, except for participating in classic calcium phosphate homeostasis and bone metabolism. One study[10] suggested that a lack of maternal VD might cause a decrease in immune function, impacting on reproductive disorders. There were also studies[11,12] reporting that 45% of RSA patients had VD deficiency and that pregnant women with VD deficiency might have a higher risk of RSA. These studies all indicate a correlation between VD and RSA. However, the pathogenesis of RSA caused by VD is still unclear.

Therefore, this study conducted observational and prospective intervention studies on clinical data related to VD and RSA to explore the possible pathogenesis of RSA caused by VD, provide evidence-based bases for the prevention and treatment of RSA, improve female reproductive health and enhance the will of patients with fertility intentions.

2 | MATERIALS AND METHODS

2.1 | Materials

This study randomly selected 305 patients without spontaneous abortion (SA0), 216 patients with spontaneous abortion (SA1) and 200 patients with RSA (SA2 and above) from 1421 women of childbearing age who visited the RSA specialty clinic of Hangzhou First People's Hospital from January 2021 to June 2023 to conduct a prospective clinical study.

2.1.1 | Inclusion criteria

Patients without spontaneous abortion (SA0): (1) women of childbearing age, (2) no spontaneous abortion, (3) individuals with a history of full-term healthy pregnancy or first-time non-pregnant health.

Patients with a spontaneous abortion (SA1): (1) women of childbearing age, (2) one spontaneous abortion.

Patients with RSA (SA2 and above): (1) women of childbearing age, (2) the same sexual partner had two or more consecutive spontaneous abortions.

2.1.2 | Exclusion criteria

(1) Patients with chromosomal abnormalities in RSA couples, such as balanced translocation.
(2) Patients who took VD preparations or related VD supplements within 3 months before treatment.
(3) Patients with a combination of certain malignant tumors and severe mental illness.

All research subjects signed informed consent forms, and this study was approved by the Ethics Committee of Hangzhou First People's Hospital (approval number: IIT-20230808-0172-01).

2.2 | Methods

Relevant clinical data of the research subjects were collected, including general information such as the patient's age, height, weight and reproductive history. Auxiliary laboratory tests, such as the parameter of uterine artery resistance S/D, embryonic chromosomes tested voluntarily, the content of plasma VD and the level of anticardiolipin antibody (ACA), were measured. Moreover, intervention measures of VD supplementation for VD deficiency or insufficiency and follow-up on pregnancy outcomes were performed. The specific research methods were as follows:

(1) Monitoring the dynamics of the uterine artery

Monitoring indicators: ratio of peak systolic to end diastolic flow velocity (S/D) of bilateral umbilical arteries.

Monitoring time: Unpregnant patients were in the mid-luteal phase (5–7 days after ovulation), while pregnant patients were at 10 weeks of pregnancy.

Monitoring methods: Color Doppler ultrasound (SIEMENSG60, Germany) equipped with a 3.5–7.0 MHz transvaginal microconvex probe was used to observe bilateral S/D.

Monitoring frequency: dynamic monitoring.

Reference indicators: If the bilateral S/D of the monitored person is greater than 10.5, the uterine artery resistance is high.

(2) Detecting embryonic chromosomes

Detection methods: SNP gene chips were used to detect embryonic chromosomes in spontaneous abortion; the genomic DNA of aborted embryo tissue was extracted, whole-genome chromosome detection was performed, and whether the corresponding fragment or site was abnormal was determined.

Reference indicators: chromosome number abnormality is aneuploidy and polyploid, and its structural abnormality is the change in copy number.

(3) Monitoring VD content and ACA level

Monitoring methods: VD content was detected by liquid chromatography-tandem mass spectrometry using DISIGNS reagent, and ACA levels were detected by enzyme-linked immunosorbent assay using EUROIMMUN reagent.

Monitoring frequency: dynamically monitor plasma VD, antiphospholipid antibodies and lupus anticoagulants at least twice every two months before and after treatment, and monitor early, mid, and late pregnancy each once after conception.

Reference indicators: VD > 30 ng/mL is VD deficiency, 20—30 ng/mL is VD insufficiency, and < 20 ng/mL is VD sufficiency; ACA > 20 XPL is positive, 9.4-20 XPL is weakly positive, and < 9.4 XPL is negative.

(4) Intervention methods

Supplementation with VD preparations and moderate intensity exercise in outdoor sunlight should be provided to individuals who have VD deficiency or insufficiency while improving unhealthy lifestyles and emotions. (Moderate-intensity exercise: Taking a brisk walk of 100 m per minute as an example, each exercise achieves an increase in heart rate and slight sweating.)

The research data were statistically analyzed by SPSS 20.0. The measurement data are presented as the mean ± standard deviation, and t tests and one-way ANOVA were used for intergroup comparisons. Statistical tables, line charts and bar charts were used to represent qualitative data, and the relationship between two variables was analyzed by linear regression and scatter plots.

TABLE 1 The relationship between the number of miscarriages and age in the three groups.

		Age (years) $\alpha = 0.05$	
Group	Participants (n)	1	2
0	305	30.39	
1	216	30.52	
2	200		32.13

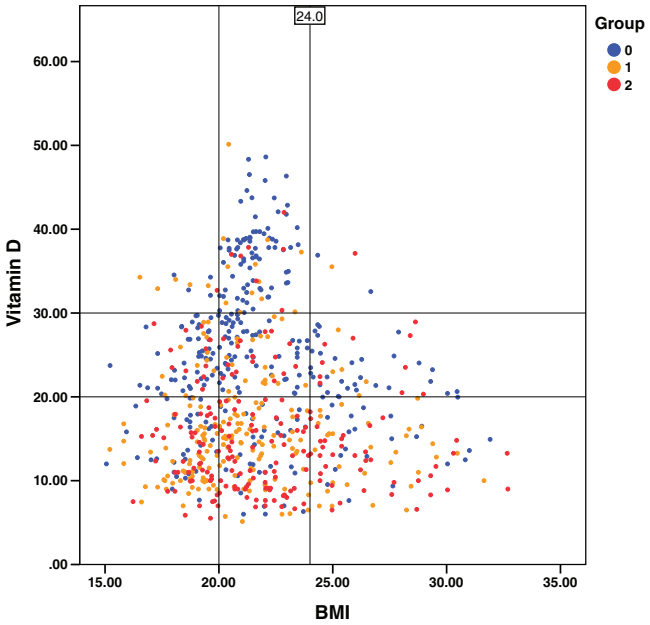

FIGURE 1 The relationship between vitamin D (ng/mL) and BMI (kg/m^2) in the three groups.

3 | RESULTS

3.1 | Basic clinical information

This study selected 305 SA0 patients, 216 SA1 patients and 200 RSA patients as the research subjects. Among the three groups, RSA patients were significantly older than the other two groups (Table 1). The rates of VD deficiency in SA1 and RSA patients were significantly higher than those in SA0 patients, while the VD insufficient and sufficient rates of SA0 patients were higher than those of the other two groups (Table 2). When body mass index (BMI) was < 20 or > 24 kg/m^2, the number of VD-deficient and VD-insufficient pregnancies significantly increased, especially in VD-deficient states where the number of spontaneous abortions increased (Figure 1). The bilateral S/D of VD-sufficient, VD-insufficient and VD-deficient patients gradually increased with statistical significance ($p \leq .018$) (Table 3) (excluding 88 cases of undetected uterine artery blood flow). Note: 0 represents SA0 patients, 1 represents SA1 patients and 2 represents RSA patients.

TABLE 2 Vitamin D levels in the three groups.

Group	the rate of VD deficiency (%)	the rate of VD insufficiency (%)	the rate of VD sufficiency (%)
0	29.51	43.28	27.21
1	75.46	16.20	8.33
2	76.50	19.00	4.50

TABLE 3 The relationship between vitamin D and uterine artery resistance.

Group	VD deficiency	VD insufficiency	VD sufficiency	*p*
Participants (n)	364	178	91	/
Bilateral S/D	19.66 ± 10.907	12.19 ± 8.702	9.12 ± 10.878	≤.018

TABLE 4 Embryonic chromosomes in spontaneous abortion.

Group	1 (n)	2 (n)	1+2 (n)
participants	216	200	416
chromosome tests	23	42	65
chromosome abnormality	7	29	36
numerical chromosome abnormality	7	23	30
structural chromosome abnormality	/	5	5
gene mutation	/	1	1

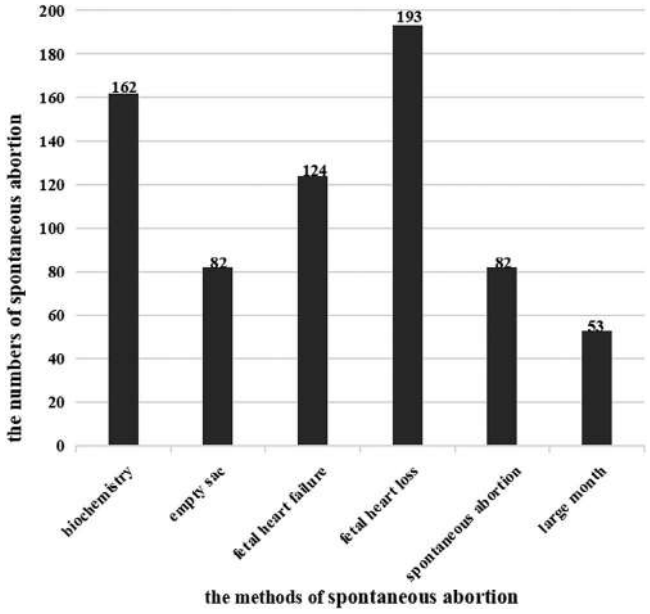

FIGURE 2 The numbers of spontaneous abortion methods in miscarriages.

3.2 | The relationship between vitamin D and pregnancy outcomes

3.2.1 | The situation of spontaneous abortion

Through observation of 721 patient cases, it was found that 15 cases of SA0 patients had threatened abortion for the first-time visit and 6 cases had first spontaneous abortion without timely correction, accounting for 15.38%. Moreover, among 416 patients with a history of one or more spontaneous abortions (SA1+RSA), there were a total of 696 spontaneous abortions with clinical manifestations including biochemical pregnancy, missed miscarriage such as empty sac, failure of fetal heart rate and loss of fetal heart rate, and spontaneous miscarriage in large months (greater than 12 weeks but less than 28 weeks) such as fetal death in the uterus, vaginal bleeding or flowing, and abdominal pain. Among them, the frequency of fetal heart loss was the highest with 193 cases; the second was biochemical pregnancy with 162 cases; and the third was fetal heart failure with 124 cases (Figure 2). Only 65 patients voluntarily underwent embryo chromosome examinations among these 416 cases. Chromosomal abnormalities accounted for 55.38% and 69.05% of RSA patients, while there were 30 cases with abnormal chromosome numbers, accounting for 83.33%, with the majority being trisomy (Table 4).

3.2.2 | The relationship between vitamin D and anticardiolipin antibody

Among the three groups of patients, abnormal ACA levels were monitored, and 186 cases were found to have a certain negative correlation between the ACA level and VD content, especially in patients with medium or low titers. After VD supplementation, the patient's VD significantly increased, while the ACA significantly decreased ($p < .001$). The main type of ACA among them was IgM, and there were also very small amounts of IgA and IgG (Table 5). The correlation coefficient (r) between VD and ACA was 0.414 and F = 76.572 ($p < .001$), which indicated that the linear model established by the two had great statistical significance. Moreover, the test for regression coefficient $t = -8.751$ ($p < .001$) indicated a negative correlation between VD(X) and ACA(Y). The correlation between the two among RSA patients was

TABLE 5 Changes in vitamin D and anticardiolipin antibody levels before and after treatment.

Group	Participants(n)	Vitamin D (ng/mL)	*t*	*p*	Anticardiolipin antibody (MPL)	*t*	*p*
before treatment	186	13.63 ± 5.101	-24.137	<.001	16.33 ± 5.487	16.638	<.001
after treatment		25.22 ± 6.530			10.98 ± 2.570		

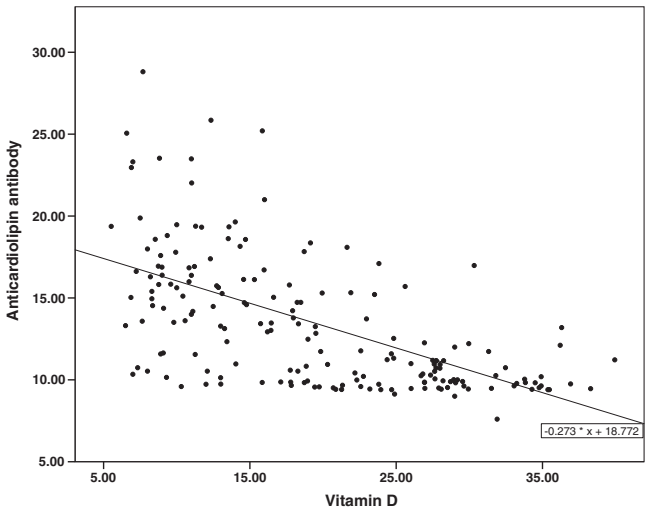

FIGURE 3 The relationship between vitamin D and anticardiolipin antibodies in RSA patients.

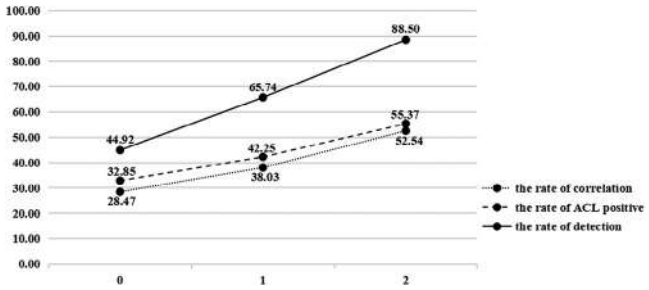

FIGURE 4 The relative levels of anticardiolipin antibodies in the three groups.

stronger (r = 0.546), and the regression equation was Y = 18.772-0.273X (Figure 3). As the number of spontaneous abortions increased, the detection rate of ACA, its positive rate and the correlation rate with VD all increased (Figure 4).

3.2.3 | Pregnancy outcomes before and after treatment

Through the analysis of 721 cases, it could be seen that the pregnancy outcomes of patients with VD supplementation significantly improved. The proportion of live births increased to 72.68%, and the clinical pregnancies and prepared pregnancies reached 13.58% and 10.26%, respectively. Importantly, adverse pregnancy outcomes accounted for only 3.48% (Table 6).

TABLE 6 Comparison of pregnancy outcomes before and after treatment.

	before treatment	after treatment
Vitamin D (ng/mL)	15.11 ± 6.072	28.53 ± 7.585
live birth (%)	6.97	72.68
clinical pregnancy (%)	3.58	13.58
preparation for pregnancy (%)	1.13	10.26
premature birth (%)	1.32	0.33
abnormal fetus (%)	0.75	0.17
biochemical pregnancy (%)	8.10	0.50
spontaneous abortion (%)	32.39	2.48
recurrent spontaneous abortion (%)	36.91	/
Sterility (%)	7.72	/
pregnancy complications (%)	1.13	/

4 | DISCUSSION

In the 721 cases of this study, the rate of VD deficiency in women without spontaneous abortion was 29.51%, and approximately 76% of patients had one or more spontaneous abortions. In addition, the probability of spontaneous abortion in women without corrected VD deficiency is 15.38%, while it is 46.88% in RSA women. These results indicate a certain relationship between VD deficiency and spontaneous abortion, which is consistent with the research conclusion of one study.[13] However, the specific pathogenesis of VD deficiency leading to spontaneous abortion is not yet clear, and possible factors include the following:

1. Compared to women without spontaneous abortion and patients with one spontaneous abortion, RSA patients are older, with a mean age of 32.13 years. This is consistent with the view that age is an independent high-risk factor for spontaneous abortion and RSA. In recent years, most studies[13-15] have found that the risk of spontaneous abortion in women over 30 years old is significantly increased in a J-shaped pattern, with a stronger association in the ages of 32 and above. Moreover, the rate of VD deficiency in RSA patients is 76.50%, and the rate is relatively high with increasing age,[16] which

may be related to changes in female hormone levels caused by age, leading to a decrease in the capacity of VD absorption.

2. When BMI is < 20 or > 24 kg/m^2, patients are more likely to be in VD-deficient or VD-insufficient states, especially in the deficient states, which are more likely to cause spontaneous abortion. These results indicate that too low or high BMI may lead to VD deficiency, or it may lead to high or low BMI due to VD deficiency, both of which are mutually causal and result in spontaneous abortion. Previous studies[17,18] have shown that women with a BMI that is too high have decreased VD bioavailability and a higher miscarriage rate, while there is a lack of relevant data on women with a BMI that is too low. BMI is the best evaluation indicator for weight management, and a BMI that is too low or high indicates that patients may have long-term unhealthy habits, leading to immune abnormalities in their bodies. This may further lead to secondary differentiation of BMI in women of childbearing age, which leads to disruption of the hypothalamic-pituitary-ovary axis and the interaction between oocyte quality and endometrial receptivity mediating embryonic abnormalities,[19,20] leading to spontaneous abortion or RSA.
3. The results of this study also suggest that the bilateral S/D of VD-sufficient, VD-insufficient and VD-deficient patients gradually increases. This indicates that there is a change in uterine artery hemodynamics in patients with VD deficiency or insufficiency, which implies that VD deficiency or insufficiency may lead to increased uterine artery resistance and insufficient perfusion of endometrial blood flow, easily leading to spontaneous abortion. The mechanism of its occurrence may be that VD deficiency weakens the ERK signaling pathway, leading to a decrease in the ability of extracellular trophoblast cells to invade the maternal decidua and reshaping spiral arteries after pregnancy,[21,22] thereby increasing uterine artery resistance, disrupting the normal intrauterine environment and affecting fetal growth.[23] Some studies[24,25] have reported that the increased resistance of uterine blood flow, which may become an independent indicator of miscarriage risk as well as an important factor leading to RSA, is associated with adverse pregnancy outcomes.
4. Among 65 patients who voluntarily underwent embryo chromosome examinations, chromosomal abnormalities accounted for 55.38% and 69.05% in RSA patients, with the majority being trisomy. These results indicate that VD deficiency or insufficiency may lead to a decline in follicle quality and nondisjunction or abnormal meiosis during fertilization, which will lead to an increase in the incidence of embryo chromosome abnormalities, further resulting in spontaneous abortion. Several studies have shown that 50–70% of spontaneous abortions involve chromosomal and genetic abnormalities,[26] which may be due to the gradual decline in ovarian reserves and oocyte integrity,[27] the production of aneuploidy in abnormal chromosome meiosis during fertilization,[28] protein defects in the polymerization checkpoint of the spindle apparatus and a decrease in cohesion protein,[29] the accumulation of mtDNA copy mutations,[30] and sperm problems.
5. Through 186 cases with ACA of medium or low titers, the study finds that there is a certain negative correlation between ACA and VD ($p < .001$), which is stronger among RSA patients. Moreover, the patient's VD significantly increased, while the ACA significantly decreased after VD supplementation ($p < .001$). These results indicate that there may be a connection between VD and ACA in which abnormalities may be more likely to result in spontaneous abortion and RSA. The results of studies[31,32] have shown that ACA is the main autoimmune antibody in the Chinese population, accounting for 53.65% of RSA patients, which could be used as a predictive indicator for spontaneous abortion in high-risk women. Moreover, a study[33] reported that insufficient VD and positive ACA in RSA patients can increase the incidence of adverse pregnancy outcomes, and there seems to be a certain connection between the two. Therefore, VD deficiency leading to spontaneous abortion may be related to the production of antiphospholipid antibodies. The mechanism by which ACA leads to RSA is relatively clear, related to PTS, mainly due to locally placental thrombosis, local inflammation and other factors inducing placental ischemic disease, ultimately leading to pathological pregnancy including RSA. However, the mechanism of action between VD and ACA may be that VD deficiency leads to abnormal ACA, and VD may reduce the levels of Autoantibody ACA by inhibiting the proliferation and activation of B cells and inducing their apoptosis to weaken the inflammatory reaction induced by ACA in trophoblastic cells under normal circumstances. In addition, this study found that the vast majority of ACAs associated with VD changes are ACA-IgM. The results of one study[34] demonstrated that ACA-IgM mainly induces early miscarriage by triggering T lymphocyte immunity.

Finally, this study also observed that the pregnancy outcomes of patients with VD supplementation significantly improved, and adverse pregnancy outcomes only accounted for 3.48%. VD supplementation can serve as a natural therapy to minimize the risk of early spontaneous abortions.[35] The way to supplement VD is to combine moderate intensity exercise outdoors with the supplementation of VD preparations. It is necessary to ensure sufficient lighting time and body exposure area because VD is mainly synthesized through skin exposure to 270–300 nm ultraviolet radiation.[36] In addition, a healthy mood and good lifestyle, such as a healthy diet, not staying up late and less drinking, should be maintained.

Overall, the rate of VD deficiency is higher in RSA patients. VD deficiency may be related to the age of women of childbearing age and a BMI that is too low or high and may lead to abnormal plasma antiphospholipid antibodies, increased uterine artery resistance and abnormal chromosomal division during fertilization, leading to spontaneous abortion and even RSA. Importantly, the improvement of VD deficiency may reduce the risk of RSA occurrence.

5 | CONCLUSION

Through a prospective design and research of clinical cases, this study finds that the improvement of VD deficiency may reduce the risk of RSA occurrence. The rate of VD deficiency is higher in RSA patients. VD

deficiency may be related to the age of women of childbearing age and a BMI that is too low or high and may lead to abnormal plasma antiphospholipid antibodies, increased uterine artery resistance and abnormal chromosomal division during fertilization. Importantly, the improvement of VD deficiency, including oral or light aerobic exercise outdoors to supplement VD, may reduce the risk of RSA occurrence. Therefore, monitoring and evaluating VD content and relevant indicators are beneficial for providing an effective basis for the early diagnosis and treatment of RSA and are of crucial significance for improving pregnancy outcomes. In addition, one spontaneous abortion should be taken seriously, and it is best to pay attention before the abortion. Although there may be occasional factors associated with one spontaneous abortion, the results of this study suggest that the vast majority of the patients have VD deficiency. The reason for VD deficiency may be related to long-term nonparticipation in outdoor aerobic exercise under sunlight. Therefore, actively screening for causes, changing unhealthy habits and improving long-term VD deficiency can prevent the recurrence of spontaneous abortion. Because RSA is a complex and diverse disease, the VD content is influenced by many factors. As a consequence, further exploration is needed to explain RSA's specific mechanism of action in future experimental research.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data is available with the permission of the corresponding author. corresponding email: 13857129395@163.com

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