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Vitamin D deficiency in an Italian cohort of infertile women

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Problem: The purpose of this study was to explore whether vitamin D might be a marker of female primary infertility in association with the presence of autoimmune diseases (ADs).

Methods: The study was a cross-sectional descriptive study in consecutive outpatients of the Polymedical Center for Prevention of Recurrent Spontaneous Abortion (RSA), in Rome, Italy. Women were eligible if they received a diagnosis of primary infertility or RSA. Serum vitamin D, calcium, and PTH were analyzed.

Results: Women with primary infertility (n=70) or RSA/non-infertile (n=105) were enrolled; controls (n=250) were included. Infertile women presented lower vitamin D (P=0.03) and higher prevalence of AD (P=0.007) than non-infertile women. In the multivariate analysis, the presence of ADs is associated with higher odds of infertility (OR=2.2), while normal vitamin D was a protective factor (OR=0.9).

Conclusion: We described that having vitamin D deficiency and suffering from an AD are independent risk factors for women primary infertility. Supplementation of vitamin D might be useful for pregnancy outcome.

KEYWORDS

autoimmunity, infertility, recurrent abortions, thyroid, vitamin D

1 | INTRODUCTION

Infertility is a common condition that affects 9%-18% of the general population, representing a complex disorder with medical, psychological, and economic aspects.[1,2] According to the Practice Committee of the American Society for Reproductive Medicine, infertility is defined as the inability to conceive a child after 12 months of regular sexual intercourse, without contraception, in couples who have never had a child. Recurrent spontaneous abortion (RSA) is defined by the occurrence of two or more consecutive failed pregnancies.[3] The causes of female infertility are different and include genetic and anatomic abnormalities as well as endocrine and autoimmune disorders (ADs).[3-6] However, unexplained etiology for female infertility accounts for almost 50% of the infertile conditions.[7,8] It is believed that a significant proportion of unexplained reproductive failure can be either directly or indirectly related to autoimmunity.[9] Evidence from the literature extensively reported the presence of autoimmunity among women with infertility/RSA.[10-13] Immune abnormalities such as the imbalance in natural killer cell (NK) levels and Th_1/Th_2 *ratio* have been associated with RSA.[14-17]

Vitamin D is a steroid hormone that, in addition to its actions on calcium and bone metabolism, exhibits a plethora of regulatory effects on immune cells.[18] Active vitamin D can work as a positive immunomodulator on both the innate and adaptive immune responses.[19] Hypovitaminosis D is highly prevalent in ADs and correlates with disease activity and comorbidities.[19] Low levels of vitamin D were also associated with increased risk of pregnancy complications such as gestational diabetes, pre-eclampsia, and fetus growth abnormality.[17] Recent data from retrospective and prospective trials have demonstrated contradictive results concerning the role of vitamin D in female reproduction and in vitro fertilization outcome.[20] In this view, the supplementation of vitamin D might be useful in women with reproductive failure and it is under investigation.[17] Serum vitamin D (25[OH]D) level

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is recognized as the best indicator of vitamin D status. The purpose of this study was to explore whether 25(OH)D levels might be a marker of female primary infertility in association with the presence of ADs.

2 | MATERIALS AND METHODS

The study was designed as a cross-sectional descriptive study in consecutive outpatients of the Polymedical Center for Prevention of RSA, San Giovanni Addolorata Hospital in Rome, Italy (time frame 2013-2015). Women were eligible for the study if they received a diagnosis of primary infertility or RSA.[3] The study population was consecutively enrolled over a period of 2 years. Among 250 women involved as possible candidate, 75 were excluded due to female factors in about 35% of cases, to male factors in 30% of cases, and to both female and male factors in 35% of cases. Additional exclusion criteria were as follows: medical history of (i) bone mineral metabolic disease, (ii) liver disease, and (iii) kidney dysfunction. Thus, 175 women with a history of primary infertility or RSA were enrolled in the study. Women with primary infertility (n=70) showed a mean infertility duration of 3±1.5 years, while RSA women (n=105) experienced two or more consecutive spontaneous abortions prior to the 20° gestational week.[3] At the time of the enrollment, all women were not pregnant and were not on vitamin D supplementation. Data on endocrine diseases as well as concurrent ADs were collected. In the whole study population, we reported cases of autoimmune thyroid diseases (ATD), including patients with positivity for antithyroid peroxidase and/or antithyroglobulin antibodies: Women with clinical thyroid dysfunction were excluded as were under treatment with levothyroxine or antithyroid drugs.[6] Moreover, we registered cases of systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), rheumatoid arthritis, and connective tissue diseases, diagnosed according to the published criteria.[4,7,9] All patients underwent laboratory examination for screening 25(OH)D, calcium, and parathormone (PTH) serum levels. A control group included women (n=250) who were age-matched with both infertile and non-infertile women. Women who served as controls referred to the Department of Laboratory Medicine (Tor Vergata University Hospital of Rome, Italy) for common laboratory investigations.

The study protocol was approved by the local ethics committee. Informed consents were obtained from the women before they entered the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was consistent with the guidelines for good clinical practice.

2.1 | Laboratory assays

All the women in the study underwent calcium, PTH, and 25(OH)D serum levels testing as part of their medical care. Women agreed to perform the panel in a context of an evaluation before conception. The venous blood (fasting) was collected under aseptic conditions, and the serum was used for the analysis of calcium, PTH, and 25(OH)D using routine laboratory techniques. Reference ranges were 8.4-10.2 mg/dL for serum calcium and 14-72 pg/mL for plasma intact PTH; 25(OH)D status was graded as deficiency (<20 ng/mL) and severe deficiency (<7 ng/mL) based on 2011 Endocrine Society guidelines.[21] Serum insulin and glucose levels were also measured to assess the HOMA (Homeostasis Model Assessment) index (Insulin [mU/mL] × Glucose [mmol/l])/22.5: Values < 2.7 were considered negative.[6,7] Body mass index (BMI, kg/m^2) was also measured.

Controls were used for their vitamin D serum levels, while laboratory reference range values were used for the "normal values" of calcium and PTH.

2.2 | Statistics

Continuous variables are presented as means±standard deviations. Before commencing statistical processing, the normality of distribution was checked with the D'Agostino-Pearson omnibus test. Normally distributed and non-normally distributed variables were compared using an independent *t* test and Mann-Whitney U test, respectively. Chi-square or Fisher's exact test was utilized for proportion comparisons. A multivariate logistic regression was carried out, correcting for confounding factors. Odds ratio (OR) was used to have a measure of association between the exposure to the variable of interest (eg, vitamin D, ADs) and the outcome (infertility). *P*-values <0.05 were considered statistically significant. All data were stored on a server, and statistical analyses were performed using SPSS Statistics version 21.0

3 | RESULTS

One hundred and seventy-five women were recruited. In this study, RSA women were considered as non-infertile women as all of them experienced the beginning of pregnancy.

Demographic data of women in the study population are summarized in Table 1.

3.1 | Vitamin D levels

Mean serum 25(OH)D resulted lower in infertile women (21.88±9.79) than in both non-infertile women (25.74±11.17, *P*=0.03) and controls (25.6±9.3, *P*=0.01), while no differences occurred between non-infertile women and controls (Figure 1A, Table 1). Mean calcium and PTH levels were in the reference range in both infertile and non-infertile groups (Table 1).

A vitamin D deficiency (<20 ng/mL) was present in a higher prevalence in the infertile group (45.7%, 32/70) than in the non-infertile one (30.4%, 32/105, *P*=0.04) and in controls (27.2%, 68/250, *P*=0.003) (Figure 1B). In these latest groups, two infertile women and one non-infertile woman showed a severe deficiency of 25(OH)D (<7 ng/mL). None of the controls had a severe deficiency of 25(OH)D.

3.2 | Autoimmune diseases

In the whole study population, 65 of 175 women were affected by one AD with 69.2% (45/65) ATD (Table 2). Infertile women showed a

TABLE 1 Data from the study groups

Parameter	Infertile group (n=70)	Non-infertile group (n=105)	Controls (n=250)	Statistical significance
Age (y)	37.57±4.35	37.01±4.33	35.6±4.8	N.S.
BMI (kg/m^2)	22±1	22.5±1	N.A.	N.S.
HOMA index	< 2.7	< 2.7	N.A.	—
25(OH)D (ng/mL)	21.88±9.79*	25.74±11.17	25.6±9.3	*<0.05
PTH (pg/mL)	43.65±17.67	44.34±22.48	N.A.	N.S.
Calcium (mg/dL)	8.10±2.36	6.28±4.22	N.A.	N.S.

BMI, body mass index; HOMA, Homeostasis Model Assessment; 25(OH)D, 25 hydroxyvitamin D; PTH, parathormone; N.S, not significant; N.A, not applicable.
Data are expressed as mean±standard deviation. Variables were compared using an independent *t* test (*P*-value <0.05 was considered significant.
*infertile vs non-infertile women, *P*=0.03, infertile women vs controls, *P*=0.01).

higher prevalence of ADs with respect to non-infertile women (52.8% vs 27.6%, *P*=0.0004, Figure 2A). In addition, ATD was prevalent in infertile group (38.6%) more than in non-infertile (17%, *P*=0.0015, Figure 2B). ATD women had no significant lower 25(OH)D levels than non-ATD women, in both the infertile and the non-infertile groups. In the whole population, all AD women had lower serum 25(OH)D than no AD women (*P*=0.04, Figure 3). This difference in 25(OH)D between women with AD and women without AD was not confirmed when women were considered distinctly in accordance with the fertility status (infertile/non-infertile).

Among the other ADs (20/65) in the whole population, we registered nine women with APS (all of them were in non-infertile group), four women with rheumatoid arthritis (75% in infertile group), six women with connective tissue diseases (83.3% in infertile group), and one woman with SLE (non-infertile woman) (Table 2).

3.3 | Univariate and multivariate analyses

The univariate analysis confirmed that the infertile group presented lower 25(OH)D levels and a higher prevalence of ADs than the non-infertile group (*P*=0.03 and *P*=0.007, respectively). At the multivariate logistic regression analysis, the presence of ADs and lower 25(OH)D levels was found to be independently associated with female infertility. The presence of at least one AD yielded an OR of 2.22 [95% confidence interval (CI) = 1.01-4.89, *P*=0.047], while higher 25(OH)D levels found to be protective factor for female primary infertility with an OR of 0.96 (95% CI = 0.92-0.99, *P*=0.041, Table 3).

4 | DISCUSSION

Although infertility among women is a multifactorial condition and might be attributed to various causes, including genetic, autoimmune, and non-autoimmune factors, in 50% of cases, the clear etiology is

TABLE 2 Autoimmune diseases in the study population

Whole population	Overall prevalence N (%)	65 (37.1)
	ATD N (%)	45/65 (69.2)
	Other N (%)	20/65 (30.8)
Infertile group	Overall prevalence N (%)	37/70 (52.8)*
	ATD N (%)	27/70 (38.6)**
	Other N (%)	10/70 (14.3)
Non-infertile group	Overall prevalence N (%)	28/105 (26.7)*
	ATD N (%)	18/105 (17.14)**
	Other N (%)	10/105 (9.5)

ATD, autoimmune thyroid diseases; Other, other autoimmune conditions (systemic lupus erythematosus, antiphospholipid syndrome, rheumatoid arthritis, and connective tissue diseases). Chi-square test was utilized for proportion comparisons (*P=0.0004; **P=0.0015).

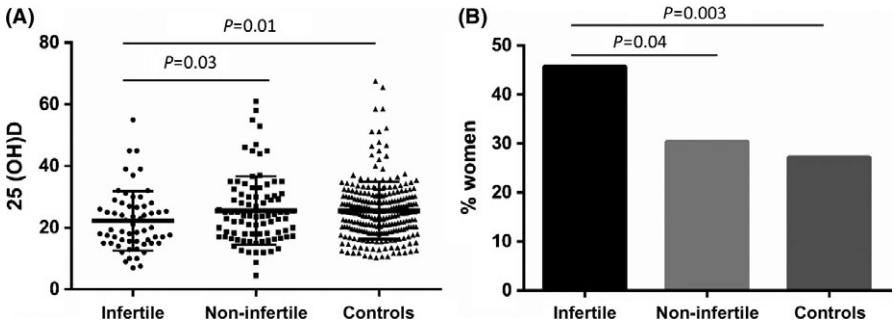

FIGURE 1 Vitamin D in the study groups. Panel A. Mean serum vitamin D (25[OH]D) resulted lower in infertile women than in non-infertile women (*P*=0.03) and in controls (*P*=0.01). Variables were compared using an independent *t* test. Panel B. Prevalence of vitamin D deficiency (<20 ng/mL) was higher in infertile women than in non-infertile (*P*=0.04) and controls (*P*=0.003). Chi-square test was utilized for proportion comparisons

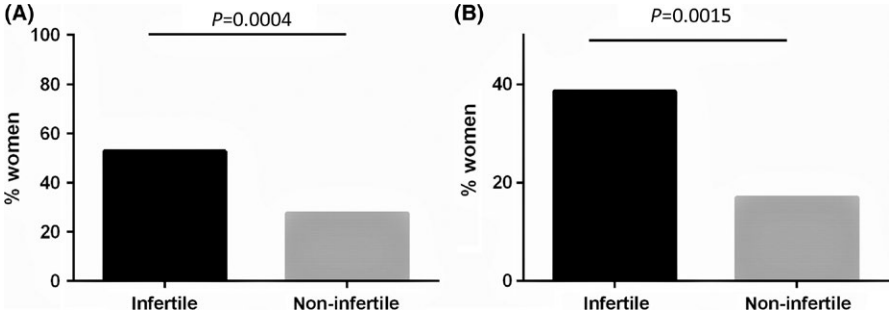

FIGURE 2 Autoimmune diseases in the study groups. Panel A. Infertile women showed a higher prevalence of autoimmune diseases (ADs) with the respect to non-infertile women (P=0.0004). Panel B. The prevalence of autoimmune thyroid diseases was higher in infertile than in non-infertile women (P=0.0015). Chi-square test was utilized for proportion comparisons

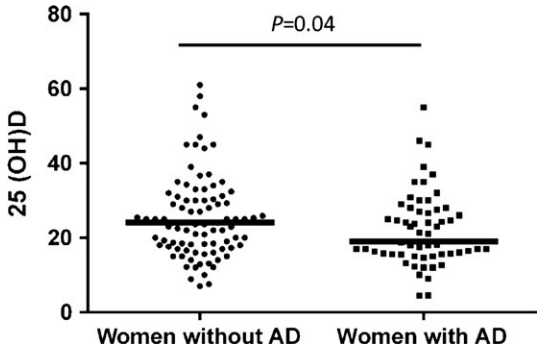

FIGURE 3 Vitamin D levels in accordance with the presence of autoimmune diseases. In the whole population, women with autoimmune diseases (ADs) had lower mean serum vitamin D (25[OH]D) than women without ADs (P=0.04). Variables were compared using an independent t test

unknown.[3-6] However, among conditions of infertility in which the etiology resulted not clear, an increased frequency of abnormalities in immune system has been reported repeatedly.[16,17,22,23] Interestingly, we have described for the first time that a deficiency in vitamin D levels was prevalent in women with primary infertility with respect to non-infertile women and controls. In this study, we decided to consider women with primary infertility as "infertile" women (without spontaneous abortion and/or living child), while women with RSA were considered as "non-infertile" women because they experienced pregnancies, spontaneous abortion, and/or living child. In the context of the primary infertility, it is possible that the vitamin D deficiency represents a potential clue of immune system dysregulation, in accordance with evidence linking vitamin D deficiency and immune system imbalance.[10,18,19] Very low vitamin D levels may thus characterize women with primary infertility according to "autoimmune mechanisms" of the pregnancy failure.[23] Similar results were reported by Al-Jaroudi et al,[24] showing a significant higher degree of vitamin D deficiency in subfertile women in comparison with controls. Pagliardini et al.[25] evaluated vitamin D levels among women requiring medical advice concerning infertility, and levels below 30 ng/mL for 89% of the entire year were observed. Moreover, in women who underwent an assisted reproduction technologies, vitamin D levels were positively related to the fertilization rate.[26] Although most of studies support the link between low levels of vitamin D and infertility, a Brazilian study has showed different results, reporting higher prevalence of vitamin D deficiency among women of reproductive age, regardless of their fertility status.[27] In another study, vitamin D levels were not associated with pregnancy outcome and did not predict the likelihood of the blastocysts implantation in women that went through euploid embryo transfer.[28] The role of vitamin D in fertility is not only reinforced by clinical and epidemiological studies but also reinforced by animal models as well. In an animal model study, female rats were divided into two groups, vitamin D-deficient and vitamin D-replete diets.[22] Among those with vitamin D deficiency, the reproductive capacity and fertility were significantly reduced in comparison to females with normal vitamin D levels regardless the calcium levels.[22] Immunoregulatory effects of vitamin D have been reported not only on T cells but also on innate immune cells.[29] Data from retrospective and prospective trials have demonstrated contradictive results concerning the role of vitamin D in female reproduction and in vitro fertilization (IVF) outcome.[20] In this view, the supplementation of vitamin D might be useful in women with reproductive failure and it is under investigation.[17] Low levels of vitamin D have been reported extensively to be associated with ADs.[18,19] In our study, however, low levels of vitamin D were found to

TABLE 3 Multivariate logistic regression analysis showing the association between the investigated variables and infertility

Variable	Coefficient	SE	Wald coefficient	P-value	OR	95% CI
Age	0 .07	0 .05	2 .06	0 .151	1.07	0 .98-1 .17
Autoimmune Diseases (yes vs not)	0 .80	0 .40	3 .96	0 .047*	2.22	1 .01-4 .89
PTH levels	−0 .00	0 .01	0 .02	0 .876	1.00	0 .98-1 .02
25(OH)D levels	−0 .04	0 .02	4 .17	0 .041*	0.96	0 .92-0 .99
Constant	−1 .83	1 .84	0 .99	0 .321		

CI, confidence interval; OR, odds ratio; PTH, parathyroid hormone (pg/mL); 25(OH)D, 25 hydroxyvitamin D (ng/mL).
*Statistically significant with P <0.05.

be independently associated with infertility as found by multivariate analysis regardless the presence of ADs (Table 3).

Women with some ADs including SLE, APS, and ATD are at increased risk of infertility.[13,19,23,30] In our study, we found that the presence of an AD is significantly associated with primary infertility in the univariate and multivariate analyses as well. The population of our study included a heterogeneous group of ADs but the highest prevalence was registered for the ATD. Assuming that infertile women have an overactive immune system, an increased prevalence of thyroid abnormalities could be expected mainly in terms of autoimmunity.[12,31] The meaning of these interesting findings was in accordance with the evidence supporting the role of thyroid function in the pathogenesis of many autoimmune disorders even in the absence of defined thyroid autoimmunity.[7,12,16,31,32] A satisfactory evidence suggests that women with infertility are more likely to have positive antithyroid antibodies than age-matched women who are not infertile, even if euthyroid.[33] In addition, the prevalence of antithyroid antibodies may be higher in women with polycystic ovarian syndrome (PCOS) than in age-matched controls.[34] Among infertile women with PCOS, the presence of antithyroid antibodies has been associated with a decreased likelihood of developing ovarian follicles in response to treatment with clomiphene citrate.[35] It has been suggested that the association between ATD and pregnancy outcome may be independent of that of non-organ-specific autoimmunity. The lower success rate of pregnancy in the patients with thyroid autoimmunity, even if also positive for antiphospholipid, further supports this hypothesis.[13]

Antiphospholipid syndrome is one of the most investigated ADs among infertile women and/or with reduced pregnancy outcome.[8,11,36-38] It can affect the obstetric outcome through various putative mechanisms, including the disruption of the feto-maternal circulation, and affect the cell division during embryogenesis and the normal function of the trophoblast.[11,36,39]

Systemic lupus erythematosus was also associated with infertility and worse prognosis in cases of SLE patients undergoing assisted reproductive techniques for pregnancy rate.[40] Additionally, higher prevalence of SLE diagnosis has been found in infertile women.[41] Menstrual irregularity is present in 53% of SLE cases under 40 years of age, and this was also associated with SLE disease activity. The function of the ovarian may be also altered in patients with SLE, leading to a decrease in the number of the ovarian reserve.[42]

Finally, our results support the link between autoimmunity and reduced reproductive capacity and suggest that the presence of any AD might affect negatively the fertility status. In our study, we did not find a significant difference of the calcium or PTH levels between infertile and non-infertile women. There are very few studies to evaluate directly the role of calcium in infertility, while, to the best of our knowledge, there are no studies to support the link between PTH levels and female infertility.[43,44] Therefore, it seems that vitamin D has a dominant role in the reproductive capacity of the female rather than calcium or PTH. Although the 95% confidence intervals (CI) of both factors, presence of AD and low levels of vitamin D, were close to one, the *P*-value was significant, and both ranges of the CI were less than one for vitamin D levels and higher than one for the presence of AD reducing the probability of reproducibility of these results in another population. Nonetheless, while our study has several strengths including its epidemiological and biostatistical design, the retrospective analysis does not allow to draw definite conclusions on the causal relationship between vitamin D and infertility. In addition, our results are expressed in terms of means concerning the aforementioned whole populations rather than in terms of comparisons between subgroups of patients with the specific reported risk factors. A subanalysis of different subpopulations is, here, not performed in accordance with the small sample size of the considered subcohorts of women. Future studies involving larger subpopulations could provide further results.

5 | CONCLUSION

ADs and low vitamin D levels are both independent risk factors for primary infertility in women. Therefore, vitamin D supplementation may be useful for improving reproductive outcomes in women suffering from primary infertility, in particular for those with concurrent ADs. However, further studies are needed to provide a clear evidentiary basis for this hypothesis.

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