ORIGINAL ARTICLE

# VITAMIN D SCREENING BEFORE FERTILITY TREATMENT PLANS: PILOT STUDY IN PCOS AND NON-PCOS INFERTILE WOMEN

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**Background:** Polycystic ovarian syndrome (PCOS) and hypovitaminosis D are two most common endocrine disorders in young women leading to many adverse metabolic consequences. Objective of this study was to compare Vitamin D (VD) levels, Body Mass Index (BMI), lipid profile, hormonal parameters, and oocytes in PCOS and non-PCOS infertile females and to explore any association of VD levels with these parameters. **Methods:** This cross-sectional study was conducted from July 2019 to June 2020 after ethical approval at Aga Khan University in collaboration with Australian Concept of Infertility Medical Centre (ACIMC). It was conducted on 88 infertile females with age range 25–45 years recruited for Intracytoplasmic Sperm Injection (ICSI). Subjects were divided into two groups; PCOS (n=37) and non-PCOS (n=51) based on diagnostic criteria of PCOS. Serum VD was analyzed using ELISA. Statistical analysis was performed on SPSS-20. Mann-Whitney U-test and Spearman's rank correlation were applied. **Results:** The mean BMI was significantly higher among PCOS as compared to non-PCOS women ($p$<0.001). There were statistically significant differences in total cholesterol, triglycerides, Low Density Lipoprotein (LDL-C), High Density Lipoprotein (HDL-C), and Very Low Density Lipoprotein (VLDL) among groups ($p$<0.05). There was significant correlation of vitamin D with maturity of oocytes ($r$=0.836, $p$<0.0001). **Conclusion:** The findings indicated that PCOs women were obese, had abnormal lipid profile with low VD levels. Low levels of VD were associated with poor maturity of oocytes which is required for successful conception.

**Keywords:** Polycystic Ovary Syndrome, PCOS, Vitamin D, deficiency, oocyte, body mass index, BMI, lipid profile

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## INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. It is classically characterized by the presence of oligo-anovulation, ovarian polycystic morphology, and hyperandrogenism.[1] Prevalence rates of PCOS are estimated to be around 4–18% worldwide and are particularly high for South Asian women, especially in Pakistani women, i.e., around 52%.[2] Classification methodologies currently being employed to serve as diagnostic criteria for PCOS include the National Institute of Health criteria, Androgen Excess and PCOS Society criteria, and the Rotterdam criteria being the most comprehensive one allowing diagnosis of non-classical phenotypical presentation of PCOS. Recently, serum anti-Müllerian hormone levels have also been suggested as a diagnostic marker for PCOS.[3]

The PCOS, being a multifactorial and heterogeneous syndrome, has a complex aetiology and its pathophysiological pathways have been under extensive research in the past decade. Vitamin D deficiency (VDD) has been identified as one of the potential risks factors.[4] In the Pakistani population, where VD status displays a staggering 53.5% VDD, it is imperative to investigate its potential association with PCOS, VDD, though very common in the general population worldwide, is even more prevalent in PCOS patients, seen in approximately 67–85%.[5] The existing literature hints towards a possible pathophysiological association involving VD metabolites affecting oocyte competence and embryo fertilization rates.[6] Research into the therapeutic effects of VD supplementation for PCOS patients has further substantiated this association.[7]

Regarding the Pakistani population, only limited work has been done till now to establish an association between VDD and PCOS, with little to no insight into the possible pathophysiological mechanisms responsible for this association. The objective of this study was to compare VD levels and relate VD levels with lipid profile, hormonal parameters and maturity of oocytes as well as Body Mass Index and in in PCOS/non-PCOS infertile women.

## METHODOLOGY

This cross-sectional study was conducted from July 2019 to June 2020 after ethical approval at Aga Khan University in collaboration with Australian Concept of Infertility Medical Centre (ACIMC).

A data collection form was filled at the time of recruitment to record clinical data such as age, height, weight, calculation of BMI, blood pressure, smoking habits, hormonal treatment, and clinical history.

A total of 88 infertile women recruited from all ethnic backgrounds for Intracytoplasmic Sperm

Injection (ICSI) with age 25–45 years after obtaining written and informed consent. Subjects were further stratified into two groups; PCOS (n=37) and non-PCOS (n=51) based on diagnosis. Women on VD therapy, calcium supplementation (for the last 6 months) or thyroxin replacement therapy were excluded. Women with diabetes, hypertension, and serious general health status were also excluded. The serum VD was analysed using Enzyme-linked immunosorbent assay kit (Cat# ab213966). The analytical sensitivity of the assay was 1.98 ng/ml, and detection range was 0.5–1010 ng/ml.

Statistical analysis was performed on SPSS-20. Mann-Whitney U-test and Spearman's rank correlation were used for comparison of results, and $p<0.05$ was considered statistically significant.

## RESULTS

A total of 88 infertile women were included in the study and were stratified further into two groups: PCOS 37 (42.1%) and non-PCOS 51 (57.9%).

The mean age of women in PCOS group was slightly higher (12.197±1.471) as compared to females in non-PCOS group (12.102±1.692), but it was not significant. The mean BMI was significantly higher among PCOS women as compared to non-PCOS women (28.966±5.227 *vs* 29.33±5.712, $p<0.001$).

Significantly low VD levels were observed in PCOS group (13.35±4.757) as compared to non-PCOS group (20.798±7.763). Number of oocytes were more in PCOS group (11.297±1.17) as compared to non-PCOS group (8.74±1.79). However, number of mature oocytes were less in PCOS group (4.0±1.17) as compared to non-PCOS group (7.78±2.4). The mean BP (Systolic and Diastolic) of women in PCOS group was slightly lower (121.243±4.734 and 75.081±3.647 respectively) as compared to non-PCOS groups (121.451±5.394 and 75.98±4.324 respectively), but it was not significant. The Follicular Stimulation Hormone (FSH), Luteinizing Hormone (LH), Anti-Müllerian Hormone (AMH), Prolactin and other parameters of women in PCOS and non-PCOS groups are shown in Table-1.

Table-2 illustrates a significant positive association of vitamin D levels with maturity of oocytes. There was a significant negative correlation of VD with all lipid parameters except HDL.

Table-3 describes correlation of VD levels with number of total oocytes (correlation coefficient, $r$= -0.0356 and $r$=0.841 in PCOS and non-PCOS) which was significant.

Figure-1 shows a significant positive correlation of both groups with maturity of oocytes ($r$=0.836, $p<0.001$). Figure-2a and b illustrate correlation of VD with number of oocytes in PCOS and non-PCOS, the correlation of both groups with maturity of oocytes is represented in Figure-3a and b.

**Table-1: Descriptive statistics of the subjects (n=88)**

| Parameters | PCOS (n=37) | Non-PCOS (n=51) | *p* |
|---|---|---|---|
| **Age** | 12.20±1.47 | 12.10±1.69 | 0.784 |
| **BMI** | 28.97±5.23 | 29.33±5.71 | 0.761 |
| **Systolic BP (mmHg)** | 121.24±4.73 | 121.45±5.39 | 0.852 |
| **Diastolic BP (mmHg)** | 75.08±3.65 | 75.98±4.32 | 0.307 |
| **FSH (mIU/mL)** | 12.22±17.54 | 6.47±2.69 | 0.023 |
| **LH (mIU/mL)** | 8.07±10.27 | 4.73±2.22 | 0.026 |
| **AMH (pmol/L)** | 2.60±2.82 | 2.14±1.48 | 0.324 |
| **Prolactin (ng/mL)** | 14.96±14.68 | 13.65±9.02 | 0.605 |
| **Estradiol (pg/mL)** | 4897.57±2587.97 | 5363.12±3600.73 | 0.504 |
| **No. of Oocytes/patients** | 11.30±1.17 | 8.75±1.80 | 0.000 |
| **No. of Mature Oocytes** | 4.03±1.66 | 7.78±2.42 | 0.000 |
| **Vitamin D (ng/ml)** | 13.35±4.76 | 20.80±7.76 | 0.000 |
| **Total Cholesterol (mg/dL)** | 223.76±17.98 | 176.20±15.02 | 0.000 |
| **Triglycerides (mg/dL)** | 210.38±40.94 | 133.71±25.02 | 0.000 |
| **HDL (mg/dL)** | 32.38±4.58 | 48.94±5.98 | 0.000 |
| **LDL (mg/dL)** | 138.32±10.11 | 114.12±16.45 | 0.000 |
| **VLDL (mg/dL)** | 46.00±7.87 | 23.82±4.76 | 0.000 |

HDL=High Density Lipoproteins, LDL=Low Density Lipoproteins, VLDL=Very low-Density Lipoproteins

**Table-2: Correlation of study variables with Vitamin-D in the subjects**

| Variables | *r* | *p* |
|---|---|---|
| **No of oocytes/patient** | 0.052 | 0.628 |
| **Oocyte maturity** | 0.836 | 0.000* |
| **Age** | -0.017 | 0.873 |
| **BMI** | 0.062 | 0.569 |
| **Systolic Blood Pressure** | -0.016 | 0.883 |
| **Diastolic Blood Pressure** | 0.001 | 0.993 |
| **Follicle Stimulating Hormone** | -0.064 | 0.553 |
| **Luteinizing Hormone** | -0.139 | 0.198 |
| **Anti-Müllerian Hormone** | -0.058 | 0.589 |
| **Prolactin** | -0.013 | 0.905 |
| **Estradiol** | 0.160 | 0.137 |
| **Total Cholesterol** | -0.427 | 0.000* |
| **Triglycerides** | -0.428 | 0.000* |
| **HDL** | 0.359 | 0.001* |
| **LDL** | -0.377 | 0.000* |
| **VLDL** | -0.410 | 0.000* |

*Significant at $p<0.01$

**Table-3: Correlation of VD with study parameters among PCOS and non-PCOS groups**

| | PCOS (n=37) | | Non-PCOS (n=51) | |
|---|---|---|---|---|
| **Parameters** | *r* | *p* | *r* | *p* |
| **No. of oocytes/patient** | -0.356 | 0.031 | 0.841 | 0.000 |
| **Oocyte maturity** | 0.784 | 0.000 | 0.792 | 0.000 |
| **Age** | -0.152 | 0.368 | -0.001 | 0.993 |
| **BMI** | 0.096 | 0.571 | 0.036 | 0.804 |
| **Systolic BP** | -0.156 | 0.355 | 0.019 | 0.894 |
| **Diastolic BP** | -0.048 | 0.778 | -0.066 | 0.644 |
| **FSH** | 0.148 | 0.381 | -0.027 | 0.852 |
| **LH** | -0.002 | 0.990 | -0.122 | 0.395 |
| **AMH** | -0.046 | 0.785 | 0.023 | 0.873 |
| **Prolactin** | 0.179 | 0.290 | -0.097 | 0.499 |
| **Estradiol** | -0.169 | 0.318 | 0.242 | 0.086 |
| **Leptin** | 0.192 | 0.256 | -0.083 | 0.563 |
| **CBC** | 0.249 | 0.137 | -0.046 | 0.748 |
| **Total Cholesterol** | -0.019 | 0.910 | -0.072 | 0.615 |
| **Triglycerides** | 0.131 | 0.441 | -0.291 | 0.038 |
| **HDL** | 0.099 | 0.559 | -0.167 | 0.243 |
| **LDL** | -0.020 | 0.908 | -0.112 | 0.435 |
| **VLDL** | -0.031 | 0.855 | 0.087 | 0.544 |

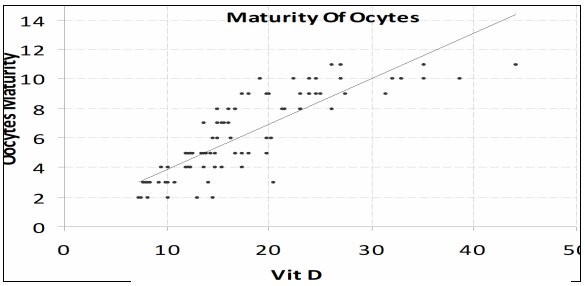


**Figure-1: Correlation of VD Levels with mature oocytes in the study population**

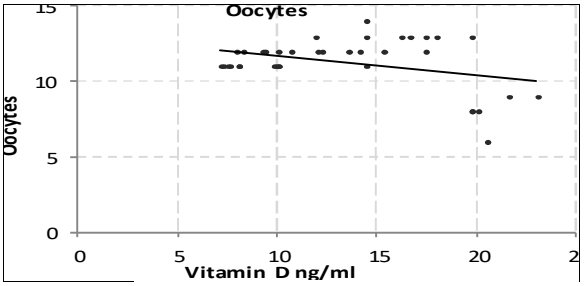


**Figure-2a: Correlation of VD Levels with number of oocytes in PCOS group**

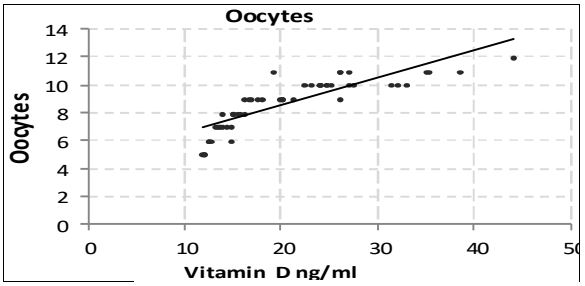


**Figure-2b: Correlation of VD Levels with number of oocytes in non-PCOS group**

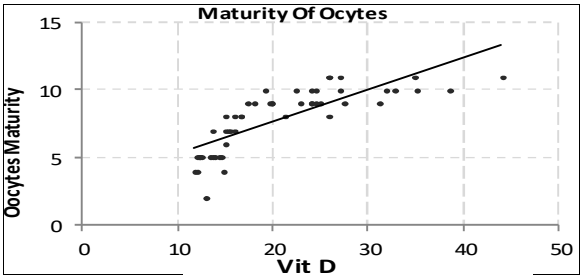


**Figure-3a: Correlation of VD Levels with maturity of oocytes in PCOS group**

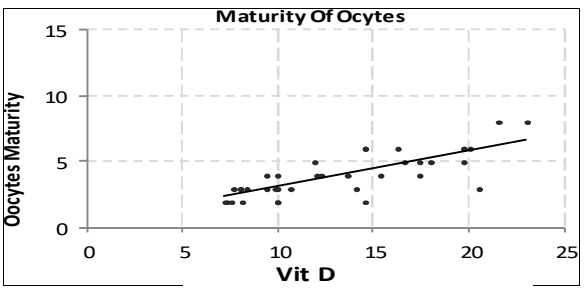


**Figure-3b: Correlation of VD Levels with maturity of oocytes in non-PCOS group**

## DISCUSSION

Our study findings of high BMI in PCOS women corroborates with some other studies[4,8]. A study in North India found a maximum number of obese women to have hirsutism and oligo ovulation and less commonly, polycystic ovaries as well. A higher BMI did not impact the LH/FSH ratio; it was comparable between PCOS women with a high BMI and those with a normal BMI.[9] Compared to PCOS-lean women, PCOS-obese women had significantly higher systolic BP, total cholesterol, LDL, and triglycerides.[10] One possible reason for relation between high BMI and PCOS is that obesity is a well-known risk factor for PCOS. PCOS is associated with higher androgen levels coupled with a reduced androgen clearance. Conversely, PCOS can also be a risk factor for obesity and higher BMI.

Women with PCOS have a higher upper-body or android obesity when compared with control women of similar BMI, the reason being that hyperandrogenism is associated with a smaller size of femoral adipocytes causing a shift of fat distribution to the upper body.[11] This android obesity then contributes to insulin resistance, hyperinsulinemia, increased risk of cardiovascular diseases and diabetes in PCOS women. Hyperandrogenism or high testosterone levels also impacts fat distribution in such a way that visceral fat increases, further impairing insulin sensitivity.[12] It is for all these reasons that weight reduction can lead to better fertility outcomes due to decreased anti-Müllerian hormone levels[13] and improvement of metabolic dysfunction in PCOS women.[14]

Our findings also suggest lower VD levels in all infertile women; however, they were significantly less in PCOS as compared to non-PCOS women. VDD is associated with ovulatory dysfunction, insulin resistance, hyperandrogenism and higher dehydroepiandrosterone sulfate (DHEAS) levels in PCOS women.[15] VD also affects lipid metabolism, so its deficiency results in lower HDL-C levels and higher LDL-C and cholesterol levels in women with PCOS.[16] Additionally, increased BMI, total cholesterol, and LDL-C, are considered risk factors of VDD in PCOS women.[17] Since PCOS women are usually obese and VD is a fat-soluble vitamin, higher body fat leads to more VD being accumulated in the fat and thus, lower serum VD levels.[18] It is for all these reasons that vitamin D supplementation can result in reduced androgen levels, improved glucose metabolism and reduced LDL-C and cholesterol levels in PCOS women.[19]

PCOS women have a reduced number of oocytes compared to non-PCOS women. According to a study[20], the participants with sufficient VD levels in the follicular fluid had a significantly greater number of retrieved oocytes compared to the participants with lower VD levels in the follicular fluid. This is explained

by evidence from other studies that VD sufficiency can result in decreased anti-Müllerian hormone levels[12], allowing for improved FSH sensitivity of growing follicles and a reduced inhibitory effect on the recruitment of growing ovarian follicles.[14] However, other studies have also stated the contrary.[7] The number of oocytes retrieved from PCOS women has been significantly higher than that in non-PCOS women, though, despite the difference in number, there was not any significant difference noted between the quality of oocytes in the two groups.[21] However, another study reported that the number of 'good quality' oocytes was lesser in the PCOS group, explaining the lower fertilization rate in those women which is in agreement with our findings except that number of oocytes were also less in our PCOS population.[10]

Our findings also indicate a significant positive correlation of VD with the maturity of oocytes. One study, upon comparing the VD deficient patients with VD sufficient patients found that the latter had better quality embryos and higher clinical pregnancy rate. Higher serum VD levels lead to better chances of ovulation.[3] VD administration induced ovulation in women before going for *in vitro* fertilization.[7] It was also noted that women successfully giving live births had significantly higher serum VD levels than those failing to do so. Lower serum VD levels also lead to higher chances of pregnancy loss.[5] However, some studies have suggested completely contradictory results. A study states that follicular fluids, which are a reliable indicator of the body's VD stores, with lower VD levels were associated with more successfully fertilized oocytes, positive hCG, better clinical pregnancy and live birth rates as compared with the follicular fluid containing higher VD levels.[22]

The negative correlation has been explained by the finding that higher follicular fluid VD levels were associated with lower glucose levels in the fluid which negatively impacted the oocyte maturation and thus, its quality.[23] The opposite correlation in our study may be due to the smaller sample size. The explanation behind the conflicting studies can be that it is only the physiological concentrations of serum VD levels that are beneficial for improving fertility by increasing endometrial receptivity. Excess VD can have detrimental effects on ovarian homeostasis, quality, and development.[21] This can be due to the ability of excess VD to antagonize the effects of other steroid sex hormones since it down-regulates oestrogen receptor (ER)-α, progesterone receptor (PR) -A and -B and steroid receptor coactivator (SRC) expression in human uterine leiomyoma cells.[24] It is because of all these reasons that VD supplementation should be done extremely cautiously by first evaluating the baseline VD levels if they are above 50 ng/mL, before any treatment for sub-fertility is started.[25]

## CONCLUSION

The findings indicated significant correlation of VD with number of total and mature oocytes as well as with BMI and all lipid parameters in PCOS and non-PCOS sub-fertile women, suggesting that VD may increase fertility through the number of mature oocytes. The possible causality of the relationship between VD and infertility deserves further investigation.

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## Contribution of Authors

**AA:** Review, editing and supervision of manuscript
**MJ:** Manuscript writing
**MA:** Manuscript statistical analysis
**MBN:** Manuscript writing
**RR:** Conceived, designed, and supervised the manuscript

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