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# VITAMIN D STATUS IN AN INFERTILE POPULATION FROM WESTERN ALGERIA

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***Key words:*** *Infertility, vitamin D status, BMI, Hormonal profile*

## INTRODUCTION

Vitamin D is well known for its function in maintaining calcium and phosphorus homeostasis and in bone mineralization (Holick, 2007). In recent years, the extraskeletal actions of vitamin D have become an important area of intensive scientific interest (Kassi, 2013). Growing evidence suggests that hypovitaminosis D is linked to an increased risk of cancer (Pilz et al, 2008), autoimmune diseases, diabetes and cardiovascular disease (Holick, 2007), (Pilz et al, 2008). al, 2008), indicating the importance of sufficient vitamin D levels. Besides, animal and human studies have shown that vitamin D is also involved in the modulation of the reproductive process in women and men (Lerchbaum et al, 2012), (Anagnostis, 2013) due to the expression of vitamin D receptor (VDR) and 1-α-hydroxylase in reproductive tissues (Lerchbaum et al, 2012), (Kinuta, 2000), (Halhali, 1991).

Infertility is a common problem that affects one in six couples. It can be defined as the inability to achieve a pregnancy after a reasonable time of sexual intercourse without contraceptive measures being taken. Evidence for changes in the prevalence of infertility is difficult to establish (Brugo-Olmedo et al, 2001). There is some evidence that in addition to the classic reproductive regulating sex steroid hormones, vitamin D also modulates reproductive processes in women and men (Lerchbaum et al, 2012) and its deficiency can impair fertility.

Based on these data, we conducted a prospective study to assess and explore the link between low vitamin D levels and fertility problems in a population of the Western Algerian region.

## MATERIALS AND METHODS

**Population:**

The study was carried out in the region of Oran (north-west of Algeria), at the level of the medically assisted procreation unit of the university hospital of Oran (EHU). All patients with fertility disorders (458 patients divided into 65 men and 393 women) who agreed to participate in our study were included.

The patients were interviewed using a comprehensive and special questionnaire developed for this purpose. Collected data were: vitamin D level, sex, age, residence, BMI, hormonal profile (FSH, LH), duration of infertility, and vitamin D supplementation.

**Statistical Analysis:**

The statistical analysis was made using SPSS 25 (Statistical Package for the Social Sciences, IBM Corporation, Chicago, IL August 2011), Chi-square Pearson test was used to compare quantitative values. The level of significance was set at 5%.

**Ethics:**

The Medical Committee of Oran University Hospital and Department of Biology, Djillali Liabes University approved the study.

## RESULTS AND DISCUSSIONS

Of a total of 458 patients, 393 cases were women (85.8%) and 65 men (14.2%) with an average age of 34.02 years (± 6.41) and extremes varying from 21 to 40 years old. The majority of cases were aged between 31-40 years (40.4%), followed by patients aged between 21-30 years with a percentage of 36% and finally patients over 40 years represented 23.6% of the studied population.

The majority of the studied cases lived in urban areas (61.1%), while 38.9% came from rural areas. The study of the population according to the BMI revealed that 44.8% presented a normal corpulence (n= 205), 9.2% presented a thinness (n= 42), 21.6% presented an overweight (n= 99) while 24.5% were obese (n=112).

Our patients had a variable infertility duration, indeed, 44.1% of cases (n=202) had an infertility duration of 13-54 months, 23.6% of cases (n=108) had a infertility duration between 55 and 96 months, while the lowest percentages were found in cases with periods of infertility of 12 months and more than 96 months with respectively (18.8% and 13.5%) (Table 1).

The study of the hormonal profile reveals that the majority of patients suffering from infertility had normal levels of the studied hormones (84.5% for

FSH, 88% for LH), while 50 patients had reduced levels of FSH (14.4%) and LH (10.9%).

Serum vitamin D [25(OH)D] assay showed that 370 cases (80.8%) had low vitamin D levels with a deficiency in 68.3% cases(<20 ng/ml), while only 88 cases (19.2%) had normal vitamin D levels (≥30ng/ml) (Table 2).

The distribution of cases according to vitamin D supplementation showed that a large percentage of patients had not taken vitamin D supplements (56.8%), while 43.2% were supplemented. However the duration of taking vitamin D supplements did not exceed one month (94.44%), and only 11 cases (5.56%) complied with vitamin supplementation for a period of 6 months (Table 2).

The study of the relationship between vitamin D level and gender showed a significant association P <0.05; while 66.67% of women were deficient in vitamin D and 14.25% had a deficiency. Furthermore, hypovitaminosis D was mainly noted in men, at 78.46%.

No significant relationship was found between age or LH hormone and Vit D level (P > 0.05). Whereas, we noticed that as age increases, the level of vitamin D decreases. Moreover, the link between residence, FSH hormone and vitamin D were significant P <0.05.

The study of the relationship between vitamin D and BMI showed that vitamin D deficiency was more noted in normal weight patients (37.7%) followed by obese (29.07%) and overweight cases (23 %). However, the normal level of vitamin D was more noted in patients with normal corpulence (80.7%). All these differences between the BMI categories in the three studied groups (in relation to the Vit D status) were statistically significant (P <0.0001).

Similarly, data analysis showed a statistically significant relationship between vitamin D status and duration of infertility P<0.0001. Indeed, the rate of vitamin D deficiency was higher in almost all categories of infertility duration: 145 cases for the infertility duration of 13-54 months, 87 cases for 55-96 months and 51 cases for longer duration of 96 months (Table 3).

Additionally, a negative association was noted between infertility duration and vitamin D status (-0.272); indicating that more vitamin D status increases, more infertility duration decreases (P <0.0001) (Figure 1).

The results of our study on infertile subjects show a high prevalence of vitamin D insufficiency, which is consistent with the result of Foray, 2012. It therefore appears that vitamin D has a positive impact on both male and female fertility. Women, with difficulty conceiving a child, usually have poor vitamin D status (Chana, 2013). This leads to prolonging the infertility duration which is similar to the results noted in our work and which revealed that the more the vitamin D status increases, the more the infertility duration decreases.

Researchers have reported a link between male fertility and vitamin D because VDR and enzymes involved in vitamin D metabolism are co-expressed in spermatids, vesicles within the epididymis, in the epithelium glandular lining of the cauda epididymis, seminal vesicles, and prostate (Blomberg et al, 2010), which explains the role of vitamin D in male reproduction.

Nevertheless, in females, VDR has been shown to be expressed in the ovaries (Agic et al, 2007), in mixed ovarian cells, and in purified granulosa cell cultures; indicating a role in sexual hormone steroidogenesis (Parikh et al, 2010).

For the relationship between vitamin D level and sex, our study revealed that there was an interaction between the two factors $P(\chi 2)<0.05$; these results are consistent with those found by (Souberbielle, 2014) who mentioned in his study that sex is considered to be one of the main non-modifiable determinants of vitamin D status.

The synthesis of vitamin D is also influenced by age (decreased production capacity); the elderly, particularly those living in institutions, represent a population at particular risk of vitamin D deficiency (low exposure, increased needs, etc.) (Holick, 2006) which confirms our results.

According to our study urbanization is considered as factor of vitamin D deficiency, living in rural areas can mean good exposure to the sun, this is similar to the results of (Holick, 2011), who considered sufficient sun exposure according to the following criteria: exposure of the legs and arms (with sun protection on the face), for 5 to 30 minutes, twice a week between ten and fifteen hours. Interestingly, vitamin D produced through the skin has a half-life approximately twice as long as ingested vitamin D (Haddad et al, 1993).

In our study, there was a significant relationship between vitamin D status and BMI, which is consistent with what other researchers have found (each increase of one unit of BMI (i.e., 1 kg/m2) is associated with a 1.15% reduction in blood vitamin D level) (Karani et al, 2013).

Obesity is a recognized independent risk factor for vitamin D deficiency.

Depending on the studies and the criteria used to define vitamin D status, the frequency of deficiency varies between 21 and 81% in obese people (Casagrande et al, 2010), (Ybarra et al, 2005) which is consistent with our series.

The link between the pituitary hormones LH and FSH and reproduction has been proven, but their relationship with vitamin D has not yet been established. Similarly, in our study we did not find a significant correlation between vitamin D and LH but we did find it with FSH.

Table 1. Demographic and laboratory data of patients infertile

| | N | Percentage % |
|---|---|---|
| **Sex** | | |
| Woman | 393 | 85,8 |
| Man | 65 | 14,2 |
| **Age** | 34.02±6.41 | |
| 21-30 | 165 | 36 |
| 31-40 | 185 | 40,4 |
| >40 | 108 | 23,6 |
| **Residence** | | |
| Rural | 178 | 38,9 |
| urban | 280 | 61,1 |
| **BMI** | 25.33±5.55 | |
| < 18,5 | 42 | 9,2 |
| 18,5-24,9 | 205 | 44,8 |
| 25-29,9 | 99 | 21,6 |
| >= 30 | 112 | 24,5 |
| **FSH level** | | |
| Low | 66 | 14,4 |
| High | 5 | 1,1 |
| normal | 387 | 84,5 |
| **LH level** | | |
| Low | 50 | 10,9 |
| High | 5 | 1,1 |
| normal | 403 | 88 |
| **Infertility duration (Months)** | 4.69±3.29 | |
| <= 12 | 86 | 18,8 |
| 13-54 | 202 | 44,1 |
| 55-96 | 108 | 23,6 |
| >96 | 62 | 13,5 |
| **Level of vitamin D** | 19.01±10.94 | |
| Insufficient | 313 | 68,3 |
| Deficit | 57 | 12,4 |
| Normal | 88 | 19,2 |
| **Vitamin D supplementation** | | |
| Yes | 198 | 43,2 |
| No | 260 | 56,8 |

According to Anagnostis et al ( 2013), special attention has been paid to the effect of vitamin D supplementation on fertility outcomes in both sexes. These data may explain our results, in which we found a significant association between vitamin D levels and supplementation because most of our patients suffered from hypovitaminodosis D.

However, at present, the recommended daily requirements of vitamin D remain under discussion and the level of evidence for non-bone aspects is insufficient. For an adult with vitamin D deficiency, Amstutz et al, (2011) proposed a loading dose of 100,000 IU of vitamin D3 every two weeks for two months (four doses) followed by 2000 IU per day (Bosomworth, 2011, Souberbielle et al, 2010, Trivedi et al, 2003). A check-up should be scheduled at three months and treatment adjusted according to the rate. The frequency of monitoring should be adapted according to the clinical elements influencing vitamin D. Unfortunately, this treatment regimen was not followed by our patients.

In order to treat the vitamin D insufficiency of our patients and since our doctors usually prescribe a 200,000 IU/ml ampoule every 15 days (two doses), we propose to continue supplementation with daily doses until the vitamin D status is balanced and give more vitamin D to those with the lowest concentrations.

Table 2. Supplementation data

| | N | Percentage % |
|---|---|---|
| Duration of supplementation | 260 | 56,8 |
| Unsupplemented | 187 | 40,8 |
| > 1 month | 0 | 0 |
| 1-3 months | 11 | 2,4 |
| 3-6 month | | |

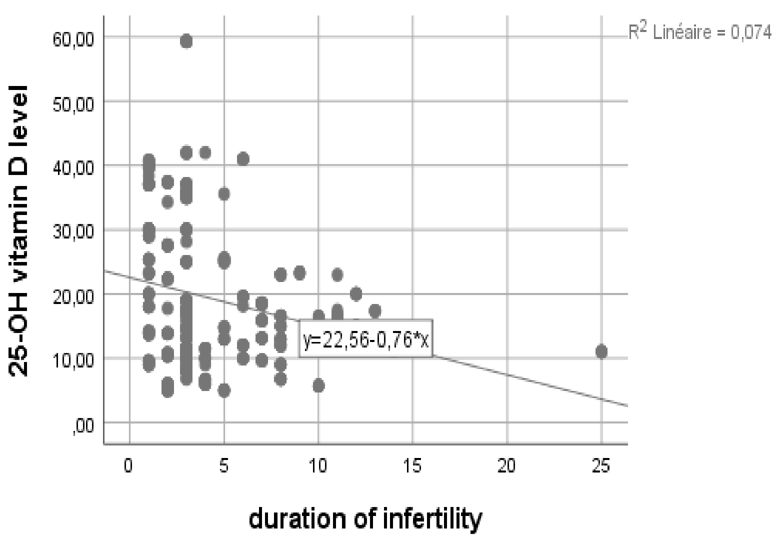


Figure 1. Correlation of vitamin D level with the duration of infertility

Table 3. Relationship of vitamin D with demographic characteristics, hormonal profile and supplementation

| | | Vitamin D status | | | P |
|---|---|---|---|---|---|
| | | Insufficient | Deficiency | Normal | |
| Sex | Woman<br>Man | 262(83.7%)<br>51(16.3%) | 56(98.2%)<br>1(1.8%) | 75(85.2%)<br>13(14.8%) | 0.015 |
| Age | Mean±SD | 34.046.14±<br>6.14 | 34.14±7.56 | 33.86±6.6 | 0.96 |
| | 21-30<br>31-40<br>>40 | 113(36.1%)<br>133(42.5%)<br>67(21.4%) | 16(28.1%)<br>26(45.6%)<br>15(26.3%) | 36(40.9%)<br>26(29.5%)<br>26(29.5%) | 0.135 |
| Area | Urbain<br>Rural | 200(63.9%)<br>113(36.1%) | 40(70.2%)<br>17(29.8%) | 40(45.5%)<br>48(54.5%) | 0.002 |
| BMI | Mean±SD | 25.52±5.70 | 25.54±6.30 | 24.55±4.36 | 0.339 |
| | < 18,5<br>18,5-24,9<br>25-29,9<br>>= 30 | 32(10.2%)<br>118(37.7%)<br>72(23%)<br>91(29.1%) | 10(17.5%)<br>16(28.1%)<br>16(28.1%)<br>15(26.3%) | 0(0%)<br>71(80.7%)<br>11(12.5%)<br>6(6.8%) | <0.0001 |
| FSH level | Low<br>High<br>normal | 55(17.6%)<br>5(1.6%)<br>253(80.8%) | 6(10.5%)<br>0(0.0%)<br>51(89.5%) | 5(5.7%)<br>0(0.0%)<br>83(84.3%) | 0.022 |
| LH level | Low<br>High<br>normal | 40(12.8%)<br>5(1.6%)<br>268(85.6%) | 5(8.8%)<br>0(0.0%)<br>52(91.2%) | 5(5.7%)<br>0(0.0%)<br>83(943%) | 0.169 |
| Infertility duration (Months) | Mean±SD | 5.42±4.16 | 4.54±3.65 | 2.19±1.37 | <0.0001 |
| | <= 12<br>13-54<br>55-96<br>>96 | 30(9.6%)<br>145(46.3%)<br>87(27.8%)<br>51(16.3%) | 15(26.3%)<br>16(28.1%)<br>15(26.3%)<br>11(19.3%) | 41(46.6%)<br>41(46.6%)<br>6(6.8%)<br>0(0.0%) | <0.0001 |
| Vitamin D supplementation | Yes<br>No | 165(52.7%)<br>148(47.3%) | 26(45.6%)<br>31(54.4%) | 7(8%)<br>81(92%) | <0.0001 |
| Duration of supplementation | Unsupplemented<br>> 1 month<br>1-3 months<br>3-6 months | 148<br>(47,3%)<br>154<br>(49,2%)<br>0 (0%)<br>11 (3,5%) | 31(54,4%)<br>26(45,6%)<br>0 (0%)<br>0 (0%) | 81(92%)<br>7 (8%)<br>0 (0%)<br>0 (0%) | <0.0001 |

## CONCLUSIONS

Whatever the considered field, it is clear that serum vitamin D concentration is a powerful biomarker of an individual's health status. Low concentrations of 25(OH)D are associated with many pathologies. In our study, we have seen that vitamin D deficiency could be associated with lower fertility given the possible relationships between vitamin D and female/ male reproduction.

Thus, the optimal levels of 25(OH) D3 for the non-classical actions in the reproductive period need to be investigated.


## ABSTRACT

There is evidence that in addition to the classical sex steroid hormones regulating reproduction, vitamin D also modulates reproductive processes in women and men and its deficiency may impair fertility.

In order to evaluate the relationship between low vitamin D status and impaired fertility, a prospective epidemiological study of 458 patients was conducted in the region of Oran (western Algeria).

The statistical analysis was performed by SPSS version 25 and Excel software, a P value < 0.05 was accepted as statistically significant.

Our study revealed that just 19.2% of our sample had normal vitamin D status and that there was no significant relationship of vitamin D status with age (P>0.05), however the results were significant with body mass indexes (P ($\chi^2$) <0.001), and with sex and residence P <0.05.

On the other hand our result indicated that as vitamin D status increased, the duration of infertility decreased (P<0.001).

The analysis of hormonal profiles showed the presence of a significance between FSH level and vitamin D deficiency (P <0.05). Moreover, we noticed that the vitamin D deficient persons are those who take the supplements (94.44%) for a period not exceeding one month.

Whatever the considered field, it is clear that serum vitamin D concentration is a powerful biomarker of an individual's health status. Low concentrations of 25(OH) D are associated with many pathologies including infertility.

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