The Effect of Serum and Follicular Fluid Vitamin D on Intracytoplasmic Sperm Injection Outcome

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Abstract
Vitamin D is a cholesterol derived, fat soluble, steroid substance present in the body. It plays an important role in the female reproductive system and regulating its functions. It is believed that vitamin D improves the endometrial thickness. The relationship between the level of vitamin D and fertilization, embryo quality and Intracytoplasmic sperm injection outcome remains controversial till now.

The aim of the study to assess the relation of vitamin D levels in serum and follicular fluid with Intracytoplasmic sperm injection outcome.

Eighty-eight women were enrolled in this study. They were classified according to the cause of infertility into case group with female factor and control group with male factor. All women were undergoing Intracytoplasmic sperm injection procedure. Serum and follicular fluid vitamin D levels were measured on the day of oocyte retrieval.

It was found that no significant differences in either serum or follicular fluid level of vitamin D between the case and control groups and higher serum and follicular fluid levels of vitamin D are associated with better pregnancy rates after Intracytoplasmic sperm injection. Further studies on vitamin D in male are required to prove its role in the Intracytoplasmic sperm injection.

The present study concluded that no significant differences in either serum or follicular fluid level of vitamin D between the case and control groups and higher serum and follicular fluid levels of vitamin D are associated with better pregnancy rates after Intracytoplasmic sperm injection.

Keywords: Vitamin D, follicular fluid and Intracytoplasmic sperm injection.

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Introduction
Vitamin D is a cholesterol derived, fat soluble, steroid substance present in the body as either ergocalciferol (D2) or cholecalciferol (D3). The majority of vitamin D is produced in vivo as D3 when skin is exposed to the sunlight. According to the American College Of Cardiology levels of Vitamin D classifies into: severe deficiency when the serum level is below (10 ng/ml), deficiency when the level is (10-20 ng/ml), insufficiency (inadequate or suboptimal, when the level is 21 – 29 ng/ml), optimal or adequate when the level is (30 ng/ml) or over ³.

Vitamin D plays an important role in the female reproductive system and regulating its functions. Its optimal levels in the body has important role in reproductive physiology as it is necessary for allowing uterine receptivity for implantation. It has been associated with endometriosis, leiomyomas and polycystic ovarian syndrome. The relationship between the level of vitamin D and Intracytoplasmic sperm injection (ICSI) outcome remains controversial till now. Some believed that low vitamin D level might have detrimental effect on the women infertility and pregnancy rate other studies showed that vitamin D deficiency did not play an important role. In spite of these differences between these studies, almost results indicated that vitamin D deficiency may bring negative effect on the outcome of Assistant Reproductive Techniques (ART) ⁷.

Also the role of vitamin D in fertilization and embryo quality is still
controversial. In women undergoing ICSI with low level of vitamin D the implantation and pregnancy rates found to be significantly lower. This because of high level vitamin D will improve the endometrial thickness so better pregnancy rates after ICSI. Additionally the endometrial cells have the ability to produce the active form of vitamin D. Regarding the level of vitamin D in follicular fluid in previous study better outcomes in ART found when the level high while other study found the level of vitamin D in follicular fluid correlates negatively and also several studies failed to find correlations.

The aim of the present study was to assess the relation of vitamin D levels in serum and follicular fluid with ICSI outcome which deals with oocytes number and quality, fertilization rate and pregnancy rate in infertile and fertile women undergoing ICSI.

Materials and methods

This is a prospective case control study which included 88 infertile couples (44 infertile women as a case group and the other 44 women as a control group with male factor infertility) undergoing controlled ovarian hyper stimulation for Intracytoplasmic sperm injection cycle in High Institute of Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrian University. The average age of women enrolled in this study ranged between 19 and 43 years.

Ethical approval was obtained and consent was obtained from patients before the start of the study.

For all women, serum and follicular fluid vitamin D levels were measured on the day of oocyte retrieval. Serum and FF obtained were estimated for vitamin D level by enzyme-linked immunosorbent assay (ELISA) technique used. The ICSI procedure was done by the clinical embryologist in charge in the IVF Lab. Embryo transfer was done by using a flexible catheter with the aid of a trans-abdominal ultrasound and without anesthesia. All patients received progesterone therapy for the luteal phase support starting from the day of oocyte retrieval until a pregnancy test was performed. The pregnancy test is performed 12-14 days after embryo transfer.

Statistical analysis

Data were analyzed using SPSS version 24 and Microsoft office Excel 2010 for estimating the effect of different factors on study parameters. Numeric variables were presented as mean± standard error of deviation, while discrete variables were presented as number and percentage. Comparison of variables was done by using the unpaired t-test, AVOVA, Yates chi square test, Fisher exact test and Pearson correlation to test the correlation.

Results

The serum level of Vitamin D: In general serum vitamin D sever deficiency found in 6 (6.80%) women, twenty nine women (32.98%) had vitamin D deficiency, forty five women (51.13%) had vitamin D insufficiency and only eight (9.09%) women with optimal level of vitamin D as shown in table 1.

Table 1. Serum level of vitamin D.

<table>
<thead>
<tr>
<th>Vitamin D Level</th>
<th>Number of women</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe Deficiency (&lt;10 ng/ml)</td>
<td>6</td>
<td>6.80%</td>
</tr>
<tr>
<td>Deficiency (10-20 ng/ml)</td>
<td>29</td>
<td>32.98%</td>
</tr>
<tr>
<td>Insufficient (20-30 ng/ml)</td>
<td>45</td>
<td>51.13%</td>
</tr>
<tr>
<td>Sufficient (≥30 ng/ml)</td>
<td>8</td>
<td>9.09%</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Comparison of serum and follicular fluid level of vitamin D between cases and control groups.

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Cases No. =44</th>
<th>Control No. =44</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. vitD</td>
<td>22.06±9.33</td>
<td>20.51±6.57</td>
<td>0.371</td>
</tr>
<tr>
<td>FF. vitD</td>
<td>17.91±5.94</td>
<td>19.9±6.66</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Comparison between level of vitamin D in case and control groups:

The mean of serum vitamin D levels at the day of oocyte retrieval ±SD of case and control group shared in this study were (22.06±9.33 and 20.51±6.57) ng/ml respectively and the follicular fluid vitamin D levels ±SD of case and control group shared in this study were (17.91±5.94 and
19.9±6.66) ng/ml respectively but no significant difference (P>0.05) between them (Table 2).

Correlation between the total number of oocytes and Serum and follicular fluid of vitamin D in both study groups:
In the case group, there was a non-significant (p>0.05) positive correlation between either the serum or follicular fluid vitamin D level with the total number of oocytes at the day of oocyte retrieval. While in the control group there was a significant positive correlation between the serum vitamin D level and the total number of oocytes (p<0.05), and non-significant negative correlation between the follicular fluid of vitamin D level and the total number of oocytes (p>0.05) as shown in table 3.

Correlation between number of metaphase II oocyte (MII) with serum and follicular fluid of vitamin D in both study groups:
In the case group, there were non-significant positive correlations between either the serum or the follicular fluid of vitamin D level and the number of metaphase II oocyte (MII) at the day of oocyte retrieval (p>0.05) while in the control group there was a non-significant negative correlation between the serum follicular fluid of vitamin D level with the number of (MII) (p>0.05) table 4.

Correlation between fertilization rate and serum and follicular fluid of vitamin D in both study groups:
There was non-significant positive correlation between the serum vitamin D level and fertilization rate (p>0.05) in case and control group and non-significant negative correlation between the follicular fluid vitamin D level and fertilization rate in both groups (p>0.05) as shown in table 5.

The pregnancy outcome:
In this study the total number of pregnant women was twenty seven, in the cases group 12 women had become pregnant while in the control group 15 women had become pregnant. The statistical analysis showed no significant (P>0.05) difference in the pregnancy outcome in both case and control groups. (table 6)

Comparison of vitamin D according to the pregnancy outcome in cases group:
The mean of serum vitamin D levels at the day of oocyte retrieval ± SD of pregnant and non-pregnant women shared in this study were (27.6±7.97 and 19.98±9.05) ng/ml respectively. The statistical analysis showed a significant difference (P<0.05) in level of serum vitamin D between pregnant and non-pregnant group. (Table 7)
While no significant difference (P>0.05) in level of follicular fluid vitamin D between pregnant and non-pregnant group (20.61±4.81 and 16.9±6.07) ng/ml respectively as shown in table 7.

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Positive No.=12</th>
<th>Negative No.=32</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. vitD</td>
<td>27.6±7.97</td>
<td>19.9±9.05</td>
<td>0.014</td>
</tr>
<tr>
<td>FF. vitD</td>
<td>20.61±4.81</td>
<td>16.9±6.07</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Table 7. Comparison of vitamin D according to pregnancy out come in cases group by unpaired t-test. No: number, SD: Standard deviation, P- value: unpaired t-test, S. vitD: serum vitamin D, FF. vitD: follicular fluid vitamin D.

**Comparison of parameters according to pregnancy out come in control group:**

The statistical analysis showed a highly significant difference (P<0.001) in level of serum vitamin D between pregnant and non-pregnant group (25.75±4.61 and 17.8±5.78) ng/ml respectively (table 13) and a significant difference (P<0.05) in level of follicular fluid vitamin D between pregnant and non-pregnant group (23.77±5.38 and 17.9±6.45) ng/ml respectively. (Table 8)

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Positive No.=15</th>
<th>Negative No.=29</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. vitD</td>
<td>25.75±4.61</td>
<td>17.8±5.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FF. vitD</td>
<td>23.77±5.38</td>
<td>17.9±6.45</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 8. Comparison of parameters according to pregnancy out come in control group. No: number, SD: Standard deviation, P- value: unpaired t-test, S. vit. D: serum vitamin D, FF. vit. D: follicular fluid vitamin D.

**Discussion**

Only eight (9.09%) women with optimal level of vitamin D despite the abundance of sunshine in Iraq that allows vitamin D synthesis throughout the year as shown in table 1. This can be explained by limited sun exposure and increase sun avoidance behaviors and use of sunscreen because of fear of skin cancer, and increased sedentary indoor lifestyles, especially among those of reproductive, obesity which need more vitamin D supplementation, cultural clothing habits (Hijab) and lack of vitamin D fortification of food by government.

There was a non- significant positive correlation in cases group between either the serum or follicular fluid vitamin D level with the total number of oocytes at the day of oocyte retrieval but in the control group there was a significant positive correlation between the serum of vitamin D level with the total number of oocytes while non- significant negative correlation with the follicular fluid of vitamin D level. This results may be due to the control group was due to male factor so the women in this group were normal and have no pathology also the number of oocytes retrieved was good. Also that vitamin D might affect the reproduction through mediating the endometrium, not the follicular or oocytes 13.

There were none-significant correlations between the serum and the follicular fluid vitamin D level with number of mature oocytes metaphase MII at the day of oocyte retrieval. The bioavailability of vitamin D within the follicle may be affect the quality of oocyte. The measurement of serum vitamin D is a reliable indicator of its availability within the ovary but the mechanisms of action how the vitamin D may influence oocyte competence in IVF remain unclear 4. So the level of vitamin D in follicular fluid may be viewed as a marker of oocyte quality.

In the case group, there were non-significant positive correlation between the serum vitamin D level and fertilization rate in both case and control groups. But a study by Ciepiela P et al in 2018 in contrast, found that women with a vitamin D deficiency had a significantly higher fertilization rate compared to women with normal vitamin D levels 4.

Also there was a non- significant negative correlation between the vitamin D level in follicular fluid and fertilization rate in both groups. While Aleyasin et al found a negative correlation between them 14.

Regarding the pregnancy in this study the statistical analysis showed a significant difference in level of serum vitamin D between pregnant and non-pregnant group in the both groups while a high significant difference in the level of follicular fluid vitamin D in control group but not significant in case group. May be the number of patients was relatively small, and any clinically important differences could not be identified in other parameters (including oocytes number and quality). Also the heterogeneity among them included causes of infertility and...
different ovarian stimulation protocols (GnRH-a long or short protocol/GnRH-ant protocol). Beside couples with male factor infertility didn’t excluded so there was the role of spermatozoa as a factor affecting embryo development.

Conclusions

The beneficial impact of high vitamin D status on IVF outcome could be attributed to the effects of vitamin D on the endometrium, since vitamin D status was not significantly associated with ovarian response parameters 10.

Acknowledgements

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Declaration of Interest

The authors report no conflict of interest.

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