

## Role of Vitamin D Supplements in Improving Fertility among Sub Fertile Couples

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### ABSTRACT

**Background:** Vitamin D is needed for calcium and phosphorus balance in body and for bone mineralization, vitamin D also plays a very important role in reproductive processes in both men and women.

**Aim:** In women of reproductive age group, to evaluate the relationship between vitamin D and fertility in women.

**Study design & duration:** Randomized controlled design from January 2014 to Feb 2017.

**Methods:** Hundred patients with sub fertility were enrolled from OPD. Patients were randomly allocated for treatment and control group. Age, body mass index, type of infertility, etiology of infertility serum vitamin D levels, parameters of sperm count (quantitative variable, sperm progressive motility, typical form quantitative variable). Krugers criteria.

**Results:** We found high prevalence of vitamin D insufficiency in our patients. Only 12 women out of 100 women (10.9%) who checked serum 25-(OH) vitamin D were sufficient. 88 Infertile women who were deficient in serum vitamin D (less than 30ng/ml) were included in this study. We treated 44 women with vitamin D supplements and 44 women received no supplementation. During the study the embryo transfer wasn't done for four patients in the control group because of having thin endometrium until the 22<sup>nd</sup> day of the cycle. Additionally, it was not done for two patients in the experimental group because of no survived embryo after warming. 82 Final analyses consist of women with completed frozen thawed embryo transfer cycles. Clinical pregnancy rate in control group women was 20% and in experimental group it was 43%.

**Conclusion:** While comparing PCOS women who took vitamin D supplements and those with deficiency of vitamin D, women with supplements have improve menstrual frequency, and have less metabolic disturbances.

**Keywords:** vitamin D, infertility, ICSI outcome.

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### INTRODUCTION

Vitamin D role is very essential in promoting bone mineralization and maintaining calcium and phosphorus levels in body<sup>1</sup>. Low vitamin D levels are associated with autoimmune related problems, cancer, diabetes, high risk cancer and cardiovascular system<sup>1,2,3</sup>. To perform normal functions a sufficient amount of vitamin D is required. Vitamin D is mandatory for normal reproductive functions. Around 15% of couples are affected from infertility. With this complex disorder, significant psychological, medical and economic aspects are linked<sup>4</sup>.

One of the chief causes of infertility among women is polycystic ovary syndrome (PCOS). Females with PCOS have insulin resistance, an ovulatory cycles and obesity. Among 40% of couples, male factor is the underlying cause of infertility<sup>5</sup>. In men, overall semen quality is getting bad. And the main culprit behind this bad semen quality is environmental factors<sup>6</sup>. While matching with WHO criteria, 20% young males have less sperm count. And among 40% of men have less sperm count which is considered optimal for fertility<sup>7</sup>. Men aging play an essential role in lowering testosterone levels<sup>8</sup>.

It has also been seen that vitamin D metabolism affects androgen levels in body<sup>9,10</sup>. The focus of this study is to relate vitamin D concentration in body and its effect on fertility.

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### MATERIAL AND METHODS

Enrolled 100 couples with sub fertility who meet inclusion and exclusion criteria presenting in Australian concept infertility medical center Garden town Lahore were selected through consecutive sampling. 12 women out of 100 women (10.9%) who checked serum 25-(OH) vitamin D were sufficient. 88 We included women with inadequate serum Vitamin D levels i.e., less than 30 mg/ml as study subjects. We treated 44 women with vitamin D supplements and 44 women received no supplementation. Patients were randomly allocated for treatment and control group by balloting method. For 6 weeks treatment group was treated with 5mg vitamin D (200,000 IU) whereas we did not give any supplements to control group. The study was performed after taking approval from the ethics committee of the institution. Written and informed consent from the subjects was taken before start of the study. The subjects who refuse to give consent were excluded from the study. Participants taking any treatment or therapy for the fertility or taking any specific diets were excluded from the study. Subjects who have been taken vitamin D or calcium supplements in less than 3 months before start of the study were also be excluded from the study. Alcoholics, smokers or subjects with renal or liver disease were excluded from the study. In this regard no particular investigations were done to rule out kidney or liver disease of all the subjects. Subjects were ruled out on the basis of history of kidney or liver disease or previous investigations if they have any of both diseases. Subject's demographic

data were recorded. Other clinical and biochemical parameters including BMI, fasting serum vitamin D levels and infertility type, factor for infertility and pregnancy rate were recorded. For continuous and categorical variables, clinical and biochemical properties between two groups were compared by using t-test and chi square test.

**RESULTS**

Less sperm count and lesser sperm motility has a link with low vitamin D levels. Its deficiency is also related to IVF outcome, especially in females. In women suffering from PCOS, truncated levels of 25-hydroxyvitamin D (25(OH) D) are serum vitamin D level as insufficient. Vitamin D levels were significantly insufficient in our patients according to results. Just 12 women out of 100 women (10.93%) who checked serum 25-(OH) vitamin D were sufficient. 88. Infertile women who have vitamin D levels less than 30ng/ml i.e. insufficient serum vitamin D are the part of this study. We treated 44 women with vitamin D supplements and 44 women received no supplementation. During the study the embryo transfer wasn't done for four patients in the control group because of having thin endometrium until the 22<sup>nd</sup> day of the cycle. Additionally, it was not done for two patients in the case group because of no survived embryo after warming. 82 Final analyses include women who completed frozen thawed embryo transfer cycles. Clinical pregnancy rate was 20% among women of control group whereas 43% was the clinical pregnancy rate among experimental group women.

Table 1: Baseline characteristics in experimental and control group

Variables	Experimental group(n=42)	Control group (n=40)	p-value
Age (years)	28.95 ± 4.4	29.06 ± 3.6	0.893
BMI(kg/m <sup>2</sup> )	24.99 ± 3.8	25.06 ± 3.2	0.928

Independent sample t test

Table 2: Baseline characteristics in experimental and control group

Variables	Experimental group (n = 42)	Control group (n=40)
<b>Infertility (p value=0.449)</b>		
Primary	38	34
Secondary	04	06
<b>Factor (p value=0.792)</b>		
ovulatory	13	9
Tubal	5	4
Male	9	11
Mixed	12	12
Unexplained	3	4

Chi square test

Table 3: Comparison of cycle characteristics between experimental and control group

Variables	Experimental group (n = 42)	Control group (n=40)
Endometrial thickness	15.20mm	0.8mm
No. of embryo transferred	02	02
<b>Embryo quality (p value=0.083)</b>		
A	25	14
B	14	22
C	03	04

Chi square test

Table 4: Comparison of Pregnancy rate between experimental and control group

Pregnancy	Experimental group (n = 42)	Control group (n = 40)
Chemical	24 (57%)	32 (80%)
Clinical Rate	18 (43%)	8 (20%)

P value=0.026\*

Chi square test

\*Significant at 0.05

Sperm mobility, sperm count, and histological oddities of ovary, uterus, testes and sperm count can be observed with it. In women with PCOS, metabolic, endocrine disturbances and obesity, low levels of hydroxyl vitamin D (25(OH) D) were found.

Its deficiency is also related to IVF outcome, especially in females. Vitamin D containing supplements, during her reproductive age leads to improvement in menstrual cycle irregularity and metabolic disturbances. Vitamin D is associated with androgen status and semen quality of men. Vitamin D containing supplements are beneficial in increasing testosterone levels.

**DISCUSSION**

Vitamin D falls under the category of steroid hormones. Skin is the source of Vitamin D precursor which is 7-dehydrocholesterol<sup>1</sup>. 7-dehydrocholesterol converts into pro vitamin D3 when skin is exposed with ultraviolet-B radiation. This ultraviolet-B radiation instantly isomerizes itself into cholecalciferol i.e. vitamin D3<sup>1</sup>. Upon its release into circulation, vitamin D binding protein (VDBP) act as carrier and vitamin D transported by this protein. Skin with sunlight exposure, generates almost 80-90% of vitamin D. Supplements as well as diet also contributes in the supply of vitamin D, but this contribution is small. Enzyme 25-hydroxylase which is present in liver is responsible for the metabolism of vitamin D. This enzyme is also very useful in determining the status of patient's vitamin D. We can divide this status into, vitamin D sufficient (marked as 30ng/ml), insufficient (20-29 ng/ml) and deficient (20ng/ml). In kidney 25(OH)D is converted into 1,25-dihydroxyvitamin D3 with the help of enzyme 1a-hydroxylase (1,25(OH)2D3). Along with kidney, many other tissues of body also contain enzyme 1a-hydroxylase. Vitamin D receptor (VDR) which is present in skeleton, reproductive tissues and parathyroid glands is very important in bringing the actions of vitamin D (1, 9). Along with this receptor, the other mode of action of Vitamin D is through its interaction with cell surface receptors via second messengers. This second pathway which usually lasts from seconds to minutes is very fast in its response<sup>1,11</sup>. Vitamin D (VDR) receptor is very abundant in various tissues in females. As these tissues contain many vitamin D receptors, so this finding shows that vitamin D actively play its role in these tissues. Women ovaries, mixed ovarian cells and purified granulose cell cultures are sites of vitamin D receptor mRNA expression<sup>12</sup>. This expression of vitamin D receptors on these tissues shows that vitamin D has a role in sex hormones synthesis. Along these sites, in human pituitary gland and endometrium also have vitamin D receptors<sup>13,14</sup>.

Extra renal synthesis of active vitamin D is possible by endometrium is possible, this fact was found by Viganò`

et al by treating endometrial cells with vitamin D and after that by measuring the levels of 1,25 (OH)2D3.

They also reported in their study that in stromal cells of human endometrium (without their cycle phase) genes of 1 $\alpha$ -hydroxylase gene expressed in their active form. And this expression particularly increases in early pregnant decidua (15). In female reproduction, vitamin D has a biological role. Up to 13% increase in progesterone, 9% increase in estradiol, 21% increase in estrone, observed in female ovarian tissue after stimulation with 1,25(OH)2D3<sup>16</sup>. Chorio-carcinoma cell lines also possess the aromatase activity of P450 which involve the catalyzing reactions that are involved in biosynthesis of estrogens. Calcitriol act as catalyst and stimulates the regulation of human chorionic gonadotropin by controlling its expression in human syncytio trophoblasts<sup>17</sup>. Placental sex steroid production also increased by calcitriol<sup>18,19</sup>. According to results of various studies calcitriol also have some other important functions such as in placenta it increases calcium transport along with enhanced lactogen expression; it also regulates the expression of HOXA 10 in stromal cells of human endometrium<sup>20,21,22</sup>. HOXA 10 expression is very crucial not only for uterus development but also for the development of endometrium.

This will ultimately lead the uterine ability to implant a fertilized egg<sup>23</sup>. When studies were conducted using the titrated vitamin D, vitamin D receptor was also present homogenates of human testicular tissue<sup>24</sup>. Human sperms also contain VDR. These receptors are present at mid piece and in nucleus<sup>25</sup>. Cauda epididymis's glandular epithelium, caput epididymis's vesicles, prostate, spermatids and seminal vesicle also contains vitamin D metabolizing enzymes and VDR, as shown by the results of recent studies (26). In reproductive tissues, vitamin D has its influence. No one knows the exact mechanism about the role of vitamin D in male reproduction. In testis cells testosterone down regulates VDR<sup>27</sup>. Androgens increase 1 $\alpha$ -hydroxylase, in cultured human osteoblasts. In vitamin D metabolism, 1 $\alpha$ -hydroxylase acts as a key enzyme<sup>30</sup>. 1,25(OH)2D3 has a link with efflux of cholesterol, phosphorylation of proteins and enhanced survival of sperms as depicted by the results of a study which investigated at molecular level, human sperms<sup>28</sup>.

So, overall sperm maturation majorly influenced by vitamin D. As vitamin D moderate the sperm survival and affect the capacitance of sperm. During capacitation energy is needed, to fulfill this energy demand, there is less energy storage and more expenditure of energy by increasing lipid metabolism. Role of 1,25(OH)2D3 has been documented in increasing the uterine weight and in inducing the decidual reaction according to results of an in vivo study. This increase in weight and hastening of decidual reaction, suggests that decidual cell differentiation which is an important step in blastocyst implantation along with degenerative changes 1,25(OH)2D3 plays a crucial role. In northern countries during long dark winters endometrial receptivity and ovulation rates also reduced<sup>29</sup>. Among healthy women, reproduction (including PCOS, steroidogenesis and IVF outcome) are affected by vitamin D according to literature evidence. Studies that explored the association of IVF outcome with vitamin D status shows the unpredictable results. According to results of a study

which included 84 infertile females as subject who were undergoing IVF. Results from this study shows that clinical pregnancy were more easily achieved by women's who have higher serum levels of 25(OH)D and there is a significant relationship was present among improved parameters of controlled ovarian hyperstimulation and vitamin D levels (29). Contrary to this finding, between serum 25(OH)D levels and follicular fluid with IVF outcomes no significant correlation was present according to results of a study by Aleyasin et al. which included 82 infertile women<sup>36</sup>.

Anifandis et al. studied 101 women, who endured IVF–intra-cytoplasmic sperm injection (ICSI) ovarian stimulation cycles<sup>30</sup>. A comparison was made in a study regarding the quality of embryos and achievement of clinical pregnancy between women with sufficient vitamin D levels (which are 30ng/ml) and women with insufficient vitamin D level (which is 20.1-30 ng/ml). Results of this study show that those women with sufficient vitamin D levels had poor quality embryos and clinical pregnancy was achieved with more difficulty than those women with inadequate vitamin D levels<sup>30</sup>. To accurately evaluate the effects of vitamin D, in women undergoing IVF, insufficient data is available. Polycystic ovary syndrome PCOS is the leading endocrine disorder among women of reproductive age. Prevalence of PCOS is 5-10%. Among the distinctive features of PCOS includes menstrual irregularity, acne and/or alopecia, hirsutism and polycystic ovaries. Vitamin D deficiency may be the main culprit for insulin resistance and among PCOS patients, metabolic syndrome<sup>34,35</sup>. In PCOS, endocrine parameters and fertility is either linked with vitamin D, it's not clear. PCOS with low 25(OH)D has been reported in many studies<sup>36,37,38</sup>. A study was conducted in Turkey which includes 100 women, in this study researchers checked the 25(OH)D levels with DHEAS and testosterone levels and the ratio of LH/FSH also checked (48). According to results of a study which were conducted on 120 PCOS patients by Hahn et al., androgen index and SHBG was correlated with 25(OH)D levels, but no significant association was reported with total testosterone, androstenedione, DHEAS, estradiol, or LH/FSH ratio<sup>39</sup>. Another study which includes 206 women, reports a correlation of SHBG and hirsutism score with vitamin D, but this study denies the correlation of vitamin D with testosterone and free testosterone<sup>40</sup>. After adjusting for BMI, the link between vitamin D and hirsutism score was significant, but when analyzing SHBG it becomes attenuated. Results of a study which includes 27 women with PCOS and 20 control women shows that low insulin sensitivity when checked by quantitative insulin was independently related with vitamin D deficiency<sup>41</sup>. Women with PCOS were more deficient in Vitamin D as compared to control group women according to an Iranian cohort study<sup>42</sup>. A study from Edinburgh, which includes 52 women, also reported similar results<sup>43</sup>. Significant difference between PCOS and 25(OH)D has been found in latter studies, in these studies PCOS women has more weight than control group women. Lower HDL-C levels and lower insulin sensitivity is associated with Vitamin D deficiency<sup>44</sup>. However, there is no full explanation of mechanism between low 25(OH)D levels and insulin resistance available. Both healthy as well as PCOS patients have

obesity, so there must be further explanation and discussion on vitamin D deficiency in PCOS<sup>45</sup>.

## CONCLUSION

Vitamin D supplement improve sperm count and motility in men. In female reproduction, including IVF outcome, Vitamin D is involved. While comparing PCOS women who took vitamin D supplements and those with deficiency of vitamin D, women with supplements have improve menstrual frequency, and have less metabolic disturbances. Direct link has been reported between vitamin D levels and semen quality as well as androgen status of men. With vitamin D supplements, testosterone levels can be boost up.

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