Vitamin D Deficiency Does Not Influence Reproductive Outcomes of IVF–ICSI: A Study of Oocyte Donors and Recipients

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ABSTRACT

Background: Vitamin D and its active metabolite, 1,25-dihydroxy vitamin D (1,25-(OH)2D3), play a significant role in reproduction. Aim: To assess the effect of serum 25-hydroxy vitamin D level on oocyte quality and endometrial receptivity by studying oocyte donors and their recipients. Materials and Methods: This prospective study consisted of two groups: Group A (recipient group) and Group B (donor group). All the participants of Groups A1 and B1 as well as Groups A2 and B2 were subcategorized into vitamin D-deficient (<20 ng/mL) and vitamin D replete-insufficient (20 to ≥30 ng/mL), respectively. Results: In the recipient group, out of the 192 participants, 123 were in A1 group, and 69 were in A2 group. In donor group, out of the 99 participants, 54 were in B1 group, and 45 in B2 group. In the recipient group, Group A2 had a higher clinical pregnancy rate, implantation rate and ongoing pregnancy rate, and a lower abortion rate as compared to that of A1, but these are statistically insignificant. The difference in endometrial thickness and number of embryos transferred between both groups was insignificant. In the donor group, the total number of days of controlled ovarian hyperstimulation, the dose of gonadotropins, the number of oocytes retrieved, the percentage of mature oocytes, and the percentage of usable embryos were higher in Group B2 than those in Group B1, but these are statistically insignificant. The fertilization rate was statistically insignificant between Groups B1 and B2. Conclusion: Vitamin D deficiency leads to lower reproductive outcomes, though not statistically significant and, thereby, does not have a negative influence on in-vitro fertilization–intracytoplasmic sperm injection outcomes.

KEYWORDS: Deficiency, in-vitro fertilization, infertility, reproduction, vitamin D

INTRODUCTION

Vitamins are necessary for metabolism, maintenance, and growth in humans. They also have specific roles to play for the proper functioning of organ systems.[1] Among all, vitamin D, a steroid hormone, is involved in various functions of body including calcium and phosphorus homeostasis, bone metabolism, cell differentiation, and proliferation.[2-4] It is metabolized by the liver to 25(OH) D, and then converted into 1,25-(OH)2D3 (1,25-dihydroxycholecalciferol, calcitriol, or active vitamin D hormone) by the kidneys. 25(OH) D has some metabolic activity, but 1,25-dihydroxy vitamin D (1,25-(OH)2D3) is the most active metabolite of vitamin D.[5]

In the recent years, low vitamin D level has become the most prevalent nutritional deficiency, which has increased the incidence of low vitamin D status worldwide.[6-8] Vitamin D deficiency has also become a national health concern in India. Although India is a tropical country with abundant sunshine, still vitamin D deficiency is a more common problem in all age groups and in both sexes across the country. Above 80% of urban Indians, including pregnant women, new born, children, young adults, and the elderly, have a vitamin D level below 20 ng/mL.[9,10]
Various factors such as age, skin pigmentation, physical activity, obesity, receiving nutrients, sunlight, and seasonal changes could also impact the blood vitamin D level.\textsuperscript{[11]}

Apart from its physiological functions, the relationship between vitamin D and reproductive function has long been recognized.\textsuperscript{[12]} According to the World Health Organization, approximately 10–25\% of the couples suffer from infertility disorder. It affects about 60–80 million couples worldwide. Infertility in women might result from many reasons including the deficiency of vitamin D.\textsuperscript{[11,13]} Recently, vitamin D has received much attention for its role in fertilization and reproduction.\textsuperscript{[14-16]} It has been suggested that vitamin D and its active metabolite, 1,25-(OH)\textsubscript{2}D\textsubscript{3}, play an important role in reproductive physiology. In the ovarian tissue, 1,25-(OH)\textsubscript{2}D\textsubscript{3} stimulates the production of progesterone, estradiol, and estrogen by 13, 9, and 21\%, respectively.\textsuperscript{[12,17,18,19]} It regulates the human chorionic gonadotropin (hCG) expression and secretion in human syncytiotrophoblast. It increases the production of placental sex steroid.\textsuperscript{[20]} It is also necessary for the adequate uterine endometrial development, allowing uterine receptivity for implantation.\textsuperscript{[21,22]} Therefore, its optimal levels in the body are important.\textsuperscript{[11]}

Various evidences are available on the noncalcemic effect of vitamin D in reproduction physiology, coupled with the clinical studies in women undergoing \textit{in-vitro} fertilization–intracytoplasmic sperm injection (IVF–ICSI) procedure using donor oocytes. But, the results of clinical studies are not consistent due to a variation in the clinical settings and the study design. Some other studies relatively found no significant correlation between the IVF outcomes and the vitamin D level in serum and follicular fluid. It is possible to evaluate each aspects of a reproductive cycle in IVF, starting from the follicular development to implantation; So study of donor–recipient cycles provides this information much better. The current study was conducted to assess the effect of serum 25-hydroxy vitamin D level in the women undergoing IVF–ICSI procedure using donor oocytes and their oocyte donors, to differentiate whether the effect, if any, is due to oocyte quality or endometrial receptivity.

**MATERIALS AND METHODS**

**Study design**

The present study was a prospective, cohort, observational, non-interventional clinical study conducted for a period of three months. The Institutional Ethics Review Board approved the study after reviewing the protocol, and written informed consents were obtained from the participants.

**Study population**

Women undergoing IVF–ICSI procedure using donor oocytes and their oocyte donors were included in the study. The participants with the characteristics, such as acute clinical illness, severe impairment of kidney/liver function, celiac disease, Crohn’s disease, cystic fibrosis, bariatric surgery, xeropsipigmentosa, chronic medication with antifungals, anticonvulsants, glucocorticoids, and anti-human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) drugs, primary hyperparathyroidism, chronic granulomatous disorders, complications following a major surgery or multiple accidental trauma, active malignancy, history of previous exposure to radiotherapy or chemotherapy, and refusal for enrollment in the clinical study, were excluded from the study.

The study population was categorized into two groups: Group A (recipient group: women undergoing IVF–ICSI procedure using donor oocytes) and Group B (donor group: women acting as oocyte donors).

All the participants were investigated for serum 25-hydroxy vitamin D level and categorized into two groups according to the vitamin D level (1) A1 and B1-vitamin D deficient (<20 ng/mL), and (2) A2 and B2-vitamin D replete-insufficient with a range 20 to ≥30 ng/mL [Figure 1].

![Figure 1](http://www.jhrsonline.org) Subject distribution. \(N = \) total number of participants, \(n = \) number of participants.
Vitamin D measurement
Blood samples from all the patients were obtained and transported to laboratory for analysis at room temperature; an analysis was performed on the same day on the same machine for both the groups. Total 25-hydroxy vitamin D is measured with ARCHITECT 25-OH vitamin D assay (Abbott Diagnostic, USA), which is a chemiluminescent microparticle immunoassay for the quantitative determination for 25-OH vitamin D in the human serum and plasma. The assay is designed to have an imprecision of ≤10% within laboratory (total) coefficient of variation.

Vitamin D deficiency was defined as serum 25-OH vitamin D levels <20 ng/mL in accordance with the Institute of Medicine and the Endocrine Society clinical practice guidelines.\(^ {23,24}\)

Stimulation protocol
All the participants underwent transvaginal ultrasonography on day 2 of menstrual cycle. Subsequent stimulation was as follows:

For endometrium preparation in Group A
Hormone replacement therapy was started from the second day (D2) of menstrual cycle by increasing the dose of estradiol valerate (Progynova 2 mg, Bayer Group, Germany) tablets from 4 to 8 mg per day. When endometrial lining was ≥7 mm, and the donor was ready for trigger, serum progesterone level and serum 25-OH vitamin D level were measured. Patient was taken for the study if serum progesterone was <0.5 ng/mL and vaginal micronized progesterone 400 mg twice a day added. Embryo transfer was performed on day 3 or day 5, and a maximum of two embryos were transferred. The surplus good quality embryos were transferred. The surplus good quality embryos were vitrified for future use. Serum β hCG was measured 14 days after embryo transfer. Ultrasonography was performed 7 days later, if β hCG was positive. All the patients were followed up to 12 weeks of pregnancy.

Women in Group B were stimulated from D2 of menstrual cycle with a flexible antagonist protocol using recombinant follicular stimulating hormone (FSH) (Gonal-F, Merck Serono, Switzerland). The vitamin D levels were measured on the day of starting the antagonist. When at least three follicles were 17 mm or more, gonadotropin-releasing hormone, agonist 0.2 mg (Decapeptyl prefilled syringe of 0.1 mg, Ferring, Switzerland) was used to trigger final oocyte maturation and ovulation for all donors. The oocyte retrieval was performed 35 h later under general anesthesia.

ICSI procedure was performed for all the recipients, and embryos were graded by The Spanish Association for the Study of Reproductive Biology (ASEBIR) embryo grading system.

Outcomes measured
Both the groups were analyzed for primary and secondary outcomes to assess the relationship between serum 25-OH vitamin D level and different parameters of IVF–ICSI outcomes.

In Group A, the implantation rate (IR; the ratio of the number of gestational sacs observed during sonography screening after three weeks of embryo transfer to the total number of embryos transferred) was the primary outcome measure, whereas endometrial thickness (ET), clinical pregnancy rate (CPR; the ratio of women with confirmed gestations sac/pregnancy on echographic screening to the total number of women who underwent embryo transfer), abortion rate (the ratio of the number of abortion to the total number of pregnant participants), and ongoing pregnancy rate (OPR; the ratio of the number of pregnancy continue after 12 weeks of pregnancy to the total number of women underwent embryo transfer) were the secondary outcomes measures.

In Group B, the primary outcome measured was the percentage of usable embryos (the ratio of the sum of embryos transferred plus embryos freeze to the total number of oocytes with normal fertilization). The total dose of gonadotropin used, the number of days of controlled ovarian hyperstimulation, the number of oocytes retrieved, and the percentage of mature oocytes (the ratio of mature oocytes to the total oocyte number) were the secondary outcomes. All the participants were monitored throughout the study period for adverse events (AEs) and were asked to inform to the study personnel in case of any AE.

Statistical analysis
Continuous data were reported as mean ± standard deviation (SD) and categorical as percentage (%). Statistical analysis was performed using Student’s t-test, Mann–Whitney U test, Kruskal–Wallis rank test, progression test, and two-sided chi-square test. A P value less than 0.05 (P < 0.05) was considered to be significant.

RESULTS
Study population characteristics
A total of 291 women (21–50 years) were included in the study, where Group A and Group B consisted of 192 (mean age: 36.69 ± 5.85) and 99 (mean age: 25.5 ± 2.38) women, respectively. Among Group A, 64.06% \((n=123)\) women were vitamin D deficient \((A1)\), and 35.93% \((n=69)\) were vitamin D replete-insufficient \((A2)\). In Group B, 54.55% \((n=54)\) women were vitamin D deficient \((B1)\) and 45.45% \((n=45)\) were vitamin D replete-insufficient \((B2)\). The demographic characteristics of all the participants are represented in Table 1.
In-vitro fertilization–intracytoplasmic sperm injection outcomes and pregnancy outcomes

The data of all the IVF–ICSI and pregnancy outcomes measured for both the groups are presented in Tables 2 and 3.

In the recipients group (Group A), the CPR did not significantly differ between vitamin D replete-insufficient women (A2) and vitamin D deficient women (A1) (59.42% vs. 52.85%, \(P = 0.379\)). Group A2 women exhibited better IR (41.84%) as compared to Group A1 women (41.84% vs. 36.30%), but this difference was not significant \((P = 0.298)\). The ET of Group A1 and A2 was not significant statistically (9.38 ± 1.44 mm vs. 9.32 ± 1.42 mm, \(P = 0.838\)). The number of embryos transferred was statistically similar in both the groups (1.87 ± 0.34 in Group A2 vs. 1.83 ± 0.37 in Group A1; \(P = 0.553\)). Abortion rate was found to be higher in Group A1 women as compared to Group A2 women, but this difference was statistically not significant (22.76% vs. 17.39%, \(P = 0.379\)). There is no statistical difference between the two groups for the number of embryos transferred (1 or 2, \(P = 0.550\)), and the day of embryo transfer (day 3 or day 5, \(P = 0.576\)).

In the donor group (Group B), the percentage of usable embryos was higher in vitamin D replete-insufficient donors (B2) as compared to the vitamin D deficient donors group (B1) but was not statistically significant (50.98% vs. 48.65%, \(P = 0.481\)). Both the groups required similar amount of gonadotropins for stimulation (2544.21 ± 577.94 IU in Group B1 vs. 2577.21 ± 638.21 IU in Group B2, \(P = 0.788\)). The total number of days of controlled ovarian hyperstimulation were 9.56 ± 0.74 and 9.73 ± 0.81 days, respectively, in Group B1 and Group B2, but nonsignificant between both the groups \((P = 0.258)\).

The number of oocytes retrieved and the percentage of mature oocytes were higher in Group B2 as compared to Group B1, but the difference was not statistically significant between both groups for both parameters (33.33 ± 17.45 vs. 30 ± 12.24, \(P = 0.404\); 82.03% vs. 79.69%, \(P = 0.416\)), respectively. The fertilization rate was 73.27 and 71.28% in the Groups B1 and B2, respectively, but statistically nonsignificant \((P = 0.493)\).

### Table 1: Demographic profile of women as per vitamin D level

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of vitamin D</th>
<th>Number of participants (n)</th>
<th>Mean age (years)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipients (N = 192)</td>
<td>A1–deficient (&lt;20 ng/mL)</td>
<td>123</td>
<td>36.31 ± 5.88</td>
<td>0.236(a)</td>
</tr>
<tr>
<td>(mean age: 36.69 ± 5.85)</td>
<td>A2 – replete-insufficient (20 to ≥30 ng/mL)</td>
<td>69</td>
<td>37.36 ± 5.77</td>
<td></td>
</tr>
<tr>
<td>Donor (N = 99)</td>
<td>B1 – deficient (&lt;20 ng/mL)</td>
<td>54</td>
<td>25.38 ± 2.55</td>
<td>0.599(a)</td>
</tr>
<tr>
<td>(mean age: 25.50 ± 2.38)</td>
<td>B2 – replete-insufficient (20 to ≥30 ng/mL)</td>
<td>45</td>
<td>25.64 ± 2.19</td>
<td></td>
</tr>
</tbody>
</table>

\(N\) = total number of participants, \(P\) = probability value \((P < 0.05 = \text{statistically significant})\). \(^{a}\)The \(t\) test procedure.

### Table 2: Pregnancy outcomes in recipient Group A

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>A1–deficient (&lt;20 ng/mL)</th>
<th>A2 – replete-insufficient (20 to ≥30 ng/mL)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipients (N = 192)</td>
<td>Number of participants</td>
<td>123</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial thickness (mean ± SD)</td>
<td>9.38 ± 1.44</td>
<td>9.32 ± 1.42</td>
<td>0.838(a)</td>
</tr>
<tr>
<td></td>
<td>Average embryos transferred (mean ± SD)</td>
<td>1.83 ± 0.37</td>
<td>1.87 ± 0.34</td>
<td>0.553(a)</td>
</tr>
<tr>
<td></td>
<td>Pregnancy rate</td>
<td>59.35%</td>
<td>63.77%</td>
<td>0.547(a)</td>
</tr>
<tr>
<td></td>
<td>Clinical pregnancy rate</td>
<td>52.85%</td>
<td>59.42%</td>
<td>0.379(a)</td>
</tr>
<tr>
<td></td>
<td>Implantation rate</td>
<td>36.30%</td>
<td>41.84%</td>
<td>0.298(b)</td>
</tr>
<tr>
<td></td>
<td>Abortion rate</td>
<td>22.76%</td>
<td>17.39%</td>
<td>0.379(b)</td>
</tr>
<tr>
<td></td>
<td>Ongoing pregnancy rate</td>
<td>36.59%</td>
<td>44.93%</td>
<td>0.257(b)</td>
</tr>
<tr>
<td></td>
<td>Number of embryos transferred</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>20 (16.3%)</td>
<td>9 (13%)</td>
<td>0.550(b)</td>
</tr>
<tr>
<td></td>
<td>Two</td>
<td>103 (83.7%)</td>
<td>60 (87%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day of embryo transferred</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>64 (52%)</td>
<td>33 (48%)</td>
<td>0.576(b)</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>59 (48%)</td>
<td>36 (52%)</td>
<td></td>
</tr>
</tbody>
</table>

\(N\) = total number of participants, \(P\) = probability value \((P < 0.05 = \text{statistical significant})\). \(^{a}\)The \(t\) test procedure. \(^{b}\)The chi-square test.
Donor vitamin D status. Several animal studies have shown that the low level of vitamin D affects the follicular fluid vitamin D level. A systematic review by Lerchbaum et al., in 2012, through a retrospective cohort study of 188 infertile women undergoing IVF, showed the vitamin D status and the pregnancy rate of different race. Among non-Hispanic whites, pregnancy rates declined with progressively lower levels of vitamin D while in Asians, the reverse is true possibly due to their lower IVF success. Live birth rate mirrors vitamin D status.

A systematic review by Lerchbaum et al., in 2012, suggested the association of the vitamin D level with the IVF pregnancy outcomes. They reported that the supplementation of vitamin D is a safe and effective treatment, which might have beneficial effects on human reproduction.[27]

Garbedian et al., in 2013, observed a high CPR and IR in women with sufficient vitamin D level (52.5%) as compared to the vitamin D insufficient women (34.7%), but they did not determine embryo quality score and, therefore, could not recognize whether endometrial or embryo quality affected implantation and CPR. The low level of vitamin D has been implicated as a major factor leading to poor pregnancy outcomes and infertility.[28]

Rudick et al.,[7] in 2014, conducted a retrospective study in oocyte donor and recipient cycles and observed that the low level of vitamin D in the oocyte recipients was associated with lower CPR.

In contrast to these findings, Aleyasin et al.,[15] in 2011, did not find any significant correlation between 25-(OH) D levels with biochemical or CPR in 82 infertile women undergoing IVF. Fabris et al.,[29] in 2014, in a study of the impact of circulating level of total and bioavailable serum vitamin D in egg donation recipient, showed that CPR, IR, and OPR were comparable among women with normal (>30 ng/mL) insufficient (20–30) and deficient (<20). Currently, there is insufficient evidence to recommended vitamin D supplement in the patients undergoing egg donation.

Pacis et al., in 2015, assessed the role of routine vitamin D screening and replete before assisted reproductive technology, did a systemic review. Out of 34 publications retrieved, eight were included after fulfilling the inclusion criteria. One study showed negative relationship, whereas two studies showed no association, and the remaining five concluded that the outcomes were better with vitamin D repletion.[30]

Franasiak et al.,[31] in 2015, showed that the vitamin D levels do not affect the IVF outcomes following the transfer of the euploid embryos.

### Table 3: Outcomes in donor Group B

<table>
<thead>
<tr>
<th>Variables</th>
<th>B1-deficient (&lt;20 ng/mL)</th>
<th>B2 – replete-insufficient (20 to ≥30 ng/mL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor (N = 99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of participants</td>
<td>54</td>
<td>45</td>
<td>–</td>
</tr>
<tr>
<td>Total amount of gonadotropins (mean±SD IU)</td>
<td>2544.21 ± 577.94</td>
<td>2577.21 ± 638.21</td>
<td>0.788a</td>
</tr>
<tr>
<td>Number of days of controlled ovarian hyperstimulation (mean±SD)</td>
<td>9.56 ± 0.74</td>
<td>9.73 ± 0.81</td>
<td>0.258a</td>
</tr>
<tr>
<td>Oocytes retrieved (mean±SD)</td>
<td>30.74 ± 12.24</td>
<td>33.33 ± 17.45</td>
<td>0.404a</td>
</tr>
<tr>
<td>Percentage of M2 oocyte (%)</td>
<td>79.69%</td>
<td>82.03%</td>
<td>0.416a</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>73.27%</td>
<td>71.28%</td>
<td>0.493a</td>
</tr>
<tr>
<td>Percentage of usable embryos (%)</td>
<td>48.65%</td>
<td>50.98%</td>
<td>0.481a</td>
</tr>
</tbody>
</table>

N = total number of participants, P = probability value (P < 0.05 = statistical significant). aThe t test procedure. bThe chi-square test.

**DISCUSSION**

Various studies reported the influence of vitamin D in IVF–ICSI outcomes and pregnancy outcomes with conflicting results. As per the authors’ best of knowledge, this is the first prospective study to investigate the effect of serum vitamin D levels in both donors and their recipients.

A study was conducted by Halloran et al.,[22] in 1980, which showed that the low level of vitamin D affects the mating behavior, reduces fertility rate, and impairs neonatal growth in female rats. Several animal studies have also reported that the deficiency of vitamin D results in infertility in mice.[5,25]

Ozkan et al., 2010 showed that serum and follicular fluid level of 25-OH D are highly correlated (P < 0.001) and thus levels of 25-(OH) D in body fluids are reflective of vitamins repletion status. The women with higher vitamin D level in their serum and follicular fluid are significantly more likely to achieve clinical pregnancy following IVF. He also showed significant lower level of 25-(OH) D level in black versus nonblack (P = 0.001).[12]

Anifandis et al.,[26] in 2010, reported that women with a sufficient follicular fluid vitamin D had a lower quality of embryos and were not able to achieve clinical pregnancy as compared to women with deficient follicular fluid vitamin D level.

Rudik et al., in 2012, through a retrospective cohort study in 188 infertile women undergoing IVF, showed the vitamin D status and the pregnancy rate of different race. Among non-Hispanic whites, pregnancy rates declined with progressively lower levels of vitamin D while in Asians, the reverse is true possibly due to their lower IVF success. Live birth rate mirrors vitamin D status.

A systematic review by Lerchbaum et al., in 2012, suggested the association of the vitamin D level with the IVF pregnancy outcomes. They reported that the supplementation of vitamin D is a safe and effective treatment, which might have beneficial effects on human reproduction.[27]
Lv et al., in 2016, presented a systemic review and meta-analysis on the serum vitamin D status and the IVF outcome of 134 studies. The risk for lower CPR was not significantly increased in the deficient group [relative risk ratio (RR) 0.88; 95% confidence interval (CI) 0.69–1.11]. Lower vitamin D status was associated with lower live birth rate (RR 0.76, 95% CI 0.61–0.93). There is no significant correlation between deficient serum vitamin D level and lower CPR in infertile women undergoing IVF. On the other hand, deficient vitamin D level was related to lower live birth rate.[32]

Recently, a study by van de Vijver et al.,[33] in 2016, reported that the vitamin D deficiency does not significantly impair CPR among infertile women undergoing frozen–thawed cycles.

In the present study, the authors have studied the possible effect of the vitamin D level on the oocyte quality and the endometrial receptivity by studying the oocyte donors and their recipients. In the recipients, even though reproductive parameters CPR, IR, OPR, and ET were better in replete-insufficient group versus deficient group, the difference was found not to be statistically significant.

In donor group, vitamin D replete-insufficient donor and deficient donors were statistically not significant for parameters such as dose of gonadotropins \(P = 0.788\), number of days of stimulation \(P = 0.258\), number of oocytes retrieved \(P = 0.404\), percentage of mature oocytes \(P = 0.416\), fertilization rate \(P = 0.493\), and percentage of usable embryos \(P = 0.481\). No significant difference was found in any parameters in both the recipients and the donor groups, though all parameters were higher in the vitamin D deplete-insufficient group compared to the deficient group.

The strengths of the present study were:

1. The vitamin D level sampling was done in the recipients on the day of donor egg collection and in the case of donors after 5 days of gonadotropins stimulation in contrast to most studies where the sampling was done from frozen serum retrospectively. The current study provides the actual status of the vitamin D level of all women.
2. Second, all the participants were enrolled from a single center and, therefore, racial or ethnic difference and selection bias were avoided.
3. Third, there was no difference in the outcomes when single or two embryos were transferred, or with day 3 or day 5 transfer between the two groups.
4. Fourth, to make homogenous population for study recipients with A or B grade embryos according to ASEBIR grading system were transferred; thus, the biases related to embryo quality can be eliminated.

The limitations of the current study were that only serum vitamin D level was measured, not the bioavailable vitamin D level. The major advantage of measuring the bioavailable vitamin D level was to eliminate the difference due to vitamin D binding protein. However, the study population was the same; therefore, it is impossible that it could affect the study result. Second, though homogenous, other factors such as socioeconomic class, dietary habits, and sun exposure time were not considered in the study.

**CONCLUSION**

In the present study, no statistically significant difference was observed in the reproductive outcomes in the recipient and the donor groups, though the study suggested that the recipients and the donors with replete-insufficient level of vitamin D have a better reproductive outcome compared to the vitamin D deficient groups. Though vitamin D supplement is an easy and cost-effective treatment, larger multicenter and more systemic studies are required before implementing vitamin D as routine screening and supplement.

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**Conflicts of interest**

There are no conflicts of interest.

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