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Original Article

Investigation of the Relationship between Vitamin D Levels and Oocyte Quality in Women Undergoing Intracytoplasmic Sperm Injection

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Abstract

Background: Vitamin D supports oocyte development and ovarian steroidogenesis; deficiency is linked to preeclampsia and poor oocyte quality. The purpose of the current study was to investigate the relationship between vitamin D levels and oocyte quality in women undergoing ICSI cycles.

Methods: In this case-control study, 252 women were identified from medical records and classified into high-quality (n=126) and low-quality oocyte groups (n=126). Serum vitamin D levels and anti-Müllerian hormone (AMH) were recorded. Oocyte quality was assessed by an embryologist using predefined criteria for morphology and maturity. Statistical analysis included Mann–Whitney tests and multivariable logistic regression ($p<0.05$). ROC analysis was applied to determine the optimal vitamin D cutoff, reporting AUC, sensitivity, and specificity and statistical significance was set at $p<0.05$.

Results: Patients in the high-quality oocyte group had a higher median serum vitamin D level compared with those in the low-quality group (25.2 *vs.* 21.9 *ng/ml*). Logistic regression showed that sufficient vitamin D levels (≥18 *ng/ml*) were associated with increased odds of having high-quality oocytes (OR=1.85, 95%CI: 1.07–3.22). AMH levels were also positively associated with oocyte quality (OR=1.42, 95%CI: 1.11–1.82). The ROC analysis yielded an AUC of 0.61 (95%CI: 0.53–0.68), indicating modest discriminatory ability.

Conclusion: Serum vitamin D levels are associated with oocyte quality in women undergoing ICSI cycles. While higher vitamin D levels are linked to better oocyte quality, no causal inference can be drawn from this case–control study design, and interventional studies are needed to evaluate whether correction of vitamin D deficiency improves reproductive outcomes.

Keywords: Anti-Müllerian hormone, Assisted reproductive techniques, ICSI, Female infertility, In vitro fertilization, Oocyte quality, Vitamin D.

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Introduction

Vitamin D, a steroid hormone, is obtained through skin exposure to sunlight or consumption of animal-based foods such as egg yolk and cod liver oil (1, 2). Beyond its well-known role in calcium and phosphorus metabolism and bone mineralization, vitamin D is also involved in other bodily processes, including cell proliferation, differentiation, and homeostasis (3-5).

In recent years, significant attention has been given to the role of vitamin D in women's reproductive health (6, 7). Vitamin D receptors are present in various reproductive tissues, including the ovaries, uterus, and placenta, indicating its regulatory role in reproductive physiology (5, 8). Vitamin D deficiency is associated with adverse reproductive outcomes, such as preeclampsia, fetal growth restriction, and reduced success in assisted reproductive techniques (ARTs) (8). Vitamin D particularly influences ovarian steroidogenesis, anti-Müllerian hormone (AMH) production, and oocyte development, potentially affecting oocyte quality (5). Some studies have suggested that serum or follicular levels of vitamin D may be linked to pregnancy success through in vitro fertilization (IVF), possibly due to its impact on endometrial receptivity (4, 9, 10). However, evidence regarding the role of vitamin D in oocyte quality remains inconsistent, and the precise mechanism of this relationship is not yet fully understood (11).

Vitamin D deficiency is common in women with polycystic ovary syndrome (PCOS) (65–87%) and may be associated with reduced ovarian reserve and oocyte quality (12-14). Some studies have identified vitamin D deficiency as a potential factor in the development of PCOS (15, 16). This deficiency may result from vitamin D sequestration in adipose tissue and limited sunlight exposure in some women (12). Additionally, evidence suggests that uterine fibroids or leiomyomas may be linked to low vitamin D levels, with individuals deficient in vitamin D being 2.4 times more likely to develop fibroids (17). Endometriosis, a common estrogen-dependent and inflammatory condition in women of reproductive age, may also be associated with vitamin D levels (18). This condition affects 5 to 10% of women and is characterized by the growth of endometrial tissue outside the uterus (19). Several studies indicate that low or high vitamin D levels may contribute to inflammatory changes related to endometrial receptivity and implantation (20, 21). Vitamin D is also implicated in premature ovarian failure, characterized by menopause before the age of 40 (22). A positive correlation between vitamin D levels and AMH suggests that higher vitamin D levels may help maintain ovarian reserve, thereby improving oocyte quality in ART procedures (23, 24).

Given the growing importance of ART methods such as IVF in treating infertility (25), investigating factors that influence the success of these techniques is essential. Ovarian stimulation with gonadotropins and embryo transfer are critical components of this process, but various factors, such as nutritional status, particularly vitamin D levels, affect follicular development and overall pregnancy success (26-28). Although previous studies have examined the relationship between vitamin D and assisted reproductive outcomes, the evidence regarding its direct association with oocyte quality remains inconsistent, with conflicting findings reported across different populations and study designs. Moreover, most available studies have focused on fertilization rate, embryo quality, or pregnancy outcomes, rather than oocyte morphological characteristics. Therefore, the purpose of the present case–control study was to investigate the association between serum vitamin D levels and oocyte quality in women undergoing ICSI cycles.

Methods

This case-control study was conducted at Avicenna Fertility Center affiliated to Avicenna Research Institute (ARI) between 2017 and 2021. Eligible patient records were first identified according to predefined inclusion and exclusion criteria from the source population of all women undergoing ICSI cycles during the study period. Women with low-quality oocytes were defined as cases (n=126), and controls (n=126) with high-quality oocytes were randomly selected from the same source population, matched where possible on age and BMI. Sample size was calculated based on previous studies reporting an odds ratio (OR) of approximately 1.8–2.0 for the association between vitamin D sufficiency and oocyte/embryo quality. With $\alpha=0.05$, power=80%, and a 1:1 case-to-control ratio, a minimum of 110 subjects per group was required. To compensate for potential missing data, 126 subjects per group were enrolled.

Inclusion criteria comprised age between 18 and 40 years, BMI between 18 and 35 kg/m^2, AMH levels between 1 and 4 *ng/ml*, and no prior history of IVF or known metabolic and hormonal disorders such as diabetes or thyroid dysfunction. Patients who did not meet these criteria were excluded. Data were extracted from medical records including age, BMI, baseline serum vitamin D levels (measured prior to both vitamin D supplementation and the commencement of ovarian stimulation), AMH levels, type and duration of infertility, gonadotropin dose, and number of retrieved oocytes.

Serum 25-hydroxyvitamin D levels were measured using electrochemiluminescence immunoassay (ECLIA) on Roche Cobas analyzer at the central laboratory of Avicenna Fertility Center. Vitamin D levels below 30 *ng/ml* were considered insufficient according to general clinical guidelines. Oocyte quality was assessed by an experienced embryologist based on the Istanbul consensus criteria (Alpha Scientists in Reproductive Medicine and ESHRE, 2011). Mature metaphase II (MII) oocytes were graded according to cytoplasmic morphology, granulation, perivitelline space, polar body, and zona pellucida characteristics. Oocytes with normal morphology, even granulation, and appropriate size were classified as high-quality; those with dark/granular cytoplasm, vacuoles, or significant irregularities were classified as low-quality. Patients were classified into high-quality oocyte group if ≥70% of their retrieved MII oocytes were graded as high-quality; otherwise, they were assigned to low-quality oocyte group.

Data were entered into SPSS version 26 (IBM, USA). The Kolmogorov-Smirnov test was used to assess normality. The Mann–Whitney U test was used for comparison of non-normally distributed continuous variables between the two groups. Multivariable logistic regression analysis was performed to estimate ORs with 95% confidence intervals (CIs). Backward stepwise elimination was used for model building. Variables with clinical importance or a $p<0.2$ in univariate analysis were entered into the multivariable model and statistical significance was set at $p<0.05$. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the optimal vitamin D cutoff value for predicting high-quality oocytes.

The study was conducted under the supervision of the Ethics Committee of the Islamic Azad University and the Institute for Advanced Studies in Basic Sciences (IASBS), with the ethics committee code of IR.IAU.TMU.REC.1401.095. Due to the retrospective nature of the study using existing medical records, the requirement for informed consent was waived.

Results

In this study, 252 women aged 18 to 40 years with a BMI between 18 and 35 kg/m^2 and AMH levels between 1 and 4 *ng/ml*, who were referred to Avicenna Fertility Center between 2017 and 2021, were evaluated. A comprehensive baseline comparison of demographic and clinical characteristics between the high-quality and low-quality oocyte groups is presented in table 1. All variables lacked normal distribution according to the Kolmogorov-Smirnov test. Therefore, the Mann–Whitney U test was used for between-group comparisons.

No significant differences were observed between the two groups regarding age, BMI, duration of infertility, gonadotropin dose, days of gonadotropin administration, or number of retrieved oocytes. However, women in the high-quality oocyte group had significantly higher AMH levels ($p=0.004$) and higher serum vitamin D levels ($p=0.033$) compared to the low-quality oocyte group. Table 2 demonstrates that serum vitamin D levels were significantly higher in the high-quality oocyte group than those in the low-quality group ($p=0.048$). This finding suggests a potential associa-

Table 1. Baseline characteristics of participants according to oocyte quality

Variables	Low-quality oocytes (n=126)	High-quality oocytes (n=126)	p-value*
Age (years)	32.5 (28.0–36.0)	31.0 (27.0–35.0)	0.132
BMI (kg/m^2)	24.8 (22.1–27.5)	25.3 (22.5–28.1)	0.277
AMH (*ng/ml*)	2.1 (1.4–2.8)	2.6 (1.9–3.4)	0.004
Vitamin D (*ng/ml*) median (IQR)	21.9±12.3	25.2±13.6	0.048
Duration of infertility (years)	4.0 (2.0–6.0)	3.5 (2.0–5.5)	0.616
Gonadotropin dose (*IU*)	2250 (1800–2700)	2100 (1800–2550)	0.101
Days of gonadotropin administration	9.0 (8.0–10.0)	9.0 (8.0–10.0)	0.123
Number of retrieved oocytes	8.0 (5.0–11.0)	9.0 (6.0–12.0)	0.095

*Data are presented as median (IQR) unless otherwise indicated. Vitamin D levels are presented as mean ± SD. p-values were calculated using the Mann–Whitney U test.

Table 2. Mean vitamin D levels in the two groups

Vitamin D (*ng/ml*)	Mean	SD	p-value
Low-quality oocytes	21.93	12.30	0.048
High-quality oocytes	25.17	13.62	

tion between higher vitamin D levels and improved oocyte quality.

Multivariable logistic regression analysis was performed to evaluate the independent association of vitamin D with oocyte quality. When vitamin D was entered as a continuous variable, each 1 *ng/ml* increase in serum vitamin D level was associated with a 2% increase in the odds of having high-quality oocytes (OR=1.02, 95%CI: 1.00–1.04, p=0.039). AMH was also significantly associated with the outcome (OR=1.42, 95%CI: 1.11–1.82, p=0.007).

In the final model, vitamin D was categorized based on the ROC-derived cutoff (≥18 *ng/ml*). Using backward stepwise elimination, the final multivariable logistic regression model included sufficient vitamin D level, AMH, age, and BMI. As shown in table 3, sufficient vitamin D levels (≥18 *ng/ml*) were independently associated with higher odds of high-quality oocytes (OR=1.85, 95%CI: 1.07–3.22, p=0.027). Higher AMH levels were also independently associated with better oocyte quality (OR=1.42, 95%CI: 1.11–1.82, p=0.006). Age and BMI were not significant predictors in the adjusted model. The AIC value of the final model was 343.81, indicating acceptable model fit. The categorized model was preferred due to better clinical interpretability.

Discussion

Vitamin D, as a steroid hormone, plays a significant role in biological processes, including cell growth and differentiation (31). Vitamin D after being converted to calcitriol under the influence of ultraviolet B (UVB) radiation, through its specific receptors (VDR), vit D, binds to DNA and regulates the expression of genes related to folliculogenesis and steroidogenesis (32). The finding of the present study showed that serum vitamin D levels are significantly associated with oocyte quality in women undergoing infertility treatment, with levels ≥18 *ng/ml* increasing the odds of having high-quality oocytes by 1.85 times. Previous studies have also shown that vitamin D levels in follicular fluid are associated with embryo quality and IVF success (33). However, Fabris et al. reported no association between vitamin D levels and oocyte quality, which may be due to differences in assessment methods or population characteristics (34). This finding aligns with the role of vitamin D in regulating follicular growth and improving oocyte morphology (4). Mean serum vitamin D levels were significantly higher in the high-quality oocyte group than those in the low-quality group, which may be related to the influence of vitamin D on the follicular fluid environment, a key factor in oocyte maturation (35). It should be noted that vitamin D insufficiency was defined as serum levels below 30 *ng/ml* according to established clinical guidelines. However, the cutoff value of 18 *ng/ml* reported in this study was derived from ROC curve analysis and represents the optimal study-specific threshold for discriminating between high- and low-quality oocytes. Therefore, this threshold should be interpreted as a statistical discriminator within the study population rather than a clinical definition of vitamin D sufficiency or deficiency.

The findings of this study also confirmed a positive and significant relationship between AMH levels and oocyte quality. A study by Irani et al. demonstrated that vitamin D supplementation could reduce seasonal variations in AMH and improve oocyte quality (36). Similarly, Irani et al. found that serum 25-hydroxyvitamin D levels

Table 3. Multivariable logistic regression analysis for predictors of high-quality oocytes

Variables	OR	95%CI	p-value
Sufficient vitamin D (≥18 *ng/ml*)	1.85	1.07-3.22	0.027
AMH (*ng/ml*)	1.42	1.11-1.82	0.006
Age (years)	0.96	0.91-1.02	0.22
BMI (*kg/m²*)	1.03	0.96-1.11	0.40

* The model was adjusted for variables with clinical relevance or a univariate p-value <0.20. Variable selection was performed using backward stepwise elimination. Data are presented as odds ratios (OR) with 95% confidence intervals (CI)

positively correlated with AMH, and vitamin D supplementation could mitigate seasonal AMH fluctuations, leading to improved oocyte quality. In women with PCOS and vitamin D deficiency, supplementation reduced AMH levels, likely by improving folliculogenesis (36, 37). This result is consistent with the role of AMH as an indicator of ovarian reserve and the influence of vitamin D on AMH production through VDR in granulosa cells (23).

The lack of a significant association between variables such as age, BMI, gonadotropin dose, and oocyte number with oocyte quality suggests that vitamin D and AMH may be important factors associated with oocyte quality in this study population. Although age is widely recognized as one of the most important determinants of oocyte quality and reproductive outcomes in assisted reproductive technologies, no significant association between age and oocyte quality was observed in the present study. This finding should be interpreted with caution. A possible explanation is the relatively narrow age range of the study population (18–40 years) and the application of strict inclusion criteria, particularly restricting AMH levels to 1–4 *ng/ml*. These criteria may have reduced variability in ovarian reserve and reproductive aging characteristics among participants, thereby limiting the detection of age-related differences in oocyte quality. Therefore, the absence of a significant association in our study should not be interpreted as evidence against the established effect of age on reproductive potential, but rather as a consequence of the relatively homogeneous study population.

A study by Neysanian et al. showed that women with higher vitamin D levels in serum and follicular fluid had a higher chance of pregnancy (38). Muyayalo et al. reported that only vitamin D levels in follicular fluid, not serum, were associated with embryo quality, natural fertilization, implantation rates, and clinical pregnancy (33). Dehghani et al. found that fertility rates varied across groups with different vitamin D levels (deficient, insufficient, sufficient), but no statistically significant association was observed between vitamin D levels and pregnancy (39). In a study by Mohammad Beigi et al., it was revealed that daily supplementation with 400 *IU* of vitamin D3 increased follicle size, particularly after the second treatment cycle (11). In a review, Nandi et al. reported that vitamin D deficiency reduces fertility in men, whereas evidence for its impact on women's reproductive conditions, including PCOS, endometriosis, leiomyoma, and IVF outcomes, remains inconsistent (40). Additionally, Abedi et al. found that six weeks of vitamin D supplementation before ICSI improved endometrial quality (41).

However, the present study has several limitations that warrant consideration. First, vitamin D levels were not measured in follicular fluid, which could have provided more precise information about its direct impact on the oocyte environment. Additionally, the case-control design may not fully establish causality. It is recommended that future studies adopt a cohort design and measure vitamin D levels in follicular fluid to investigate the mechanisms more thoroughly. In addition, information regarding vitamin D supplementation after baseline measurement was not consistently available in all records. Therefore, potential changes in vitamin D status during treatment could not be fully accounted for and may have introduced residual bias.

Conclusion

The findings of this study indicate a significant association between serum vitamin D levels and oocyte quality in women undergoing infertility treatment. Women with high-quality oocytes had higher vitamin D levels, and AMH levels were also positively associated with oocyte quality. However, due to the observational case–control design, no causal relationship can be inferred from these findings. Further prospective studies and randomized controlled trials are needed to determine whether improving vitamin D status can enhance oocyte quality and reproductive outcomes in women undergoing assisted reproductive treatments.

Conflict of Interest

Authors declare no conflict of interests.

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