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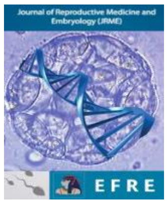

The Role of Vitamin D in Determining Intra-Cytoplasmic Sperm Injection Outcome: A Quasi-Experimental Study

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Abstract

Background: The optimization of clinical pregnancy rates following assisted reproductive technologies relies on various factors, including embryo quality and the implantation process. Recent research has focused on the role of vitamin D in enhancing implantation during intracytoplasmic Sperm Injection (ICSI).

Objective: The primary objective of this study was to investigate the impact of vitamin D level on implantation, with secondary objectives examining the associations between vitamin D levels and clinical pregnancy rates and live birth rates.

Materials and Methods: A quasi-experimental study was conducted at the Libyan National Fertility Centre and involved 60 infertile women. Each participant underwent two ICSI cycles, with the first cycle using insufficient vitamin D levels and the second using sufficient vitamin D levels. Excluded patients due to polycystic ovaries, endometriosis, premature ovarian failure, and male factor infertility. The Endocrine Society defined vitamin D insufficiency as levels <20 ng/ml.

Results: Patients were assessed at two phases based on vitamin D levels—insufficient and sufficient phase. Clinical pregnancy rates were 31.67% and 61.67%, respectively ($p<.016$). The endometrial configuration, including thickness and pattern, positively correlated with sufficient vitamin D levels ($p<.027$). However, no statistically significant difference in oocyte quality was observed.

Conclusion: The findings of this study affirm that vitamin D levels have a positive predictive value for ICSI outcomes. This study recommends vitamin D supplementation among infertile patients to enhance CPR and LBR.

Keywords: vitamin D; infertile; clinical pregnancy; live birth rates; clinical pregnancy rates; intracytoplasmic sperm injection

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Introduction

The enhancement of clinical pregnancy rates following assisted reproductive technologies is subject to multifaceted influences, including embryo quality and the implantation process. Recent studies have investigated the potential role of vitamin D in augmenting pregnancy rates, particularly in the context of intracytoplasmic sperm injection (ICSI)(1-2).

Globally, vitamin D deficiency or insufficiency has been identified in numerous societies, with existing literature attributing this deficiency to insufficient sun exposure and inadequate dietary sources of vitamin D (3). Vitamin D, a pivotal micronutrient, exerts critical functions across various physiological systems, including the immune, central nervous, cardiovascular, skeletal, and reproductive systems(4).

In the realm of reproductive health, investigations have delved into the intricate interplay between vitamin D and infertility, elucidating the distribution of its receptors in ovarian, endometrial, and placental tissues (5-7). Functioning as a hormone, vitamin D plays a significant role in steroidogenesis, implantation, and pregnancy outcomes (8- 9). Ongoing research endeavors are dedicated to establishing reference cut-off values for vitamin D in couples undergoing assisted reproductive techniques (10-12).

Notably, reproductive disorders linked to vitamin D deficiency include poor placental function, resulting in compromised embryo implantation and consequential adverse outcomes such as pregnancy loss and fetal growth restriction (13-14). Furthermore, vitamin D is implicated in calcium transport within the placenta and in gene expression associated with uterine decasualization, which is crucial in preparing for the implantation process (14).

Conversely, research has revealed a negative correlation between oocyte and embryo quality and vitamin D level in follicular fluid, indicating that lower vitamin D concentrations are associated with higher pregnancy rates (15).

Despite various studies examining the impact of vitamin D levels on the clinical outcomes of ICSI cycles, conflicting results persist, with some attributing discrepancies to confounding factors among study participants (16-17).

This study aimed to assess vitamin D levels among infertile couples using a novel methodology designed to mitigate confounding factors. This involves performing ICSI cycles after correcting vitamin D levels in patients who previously underwent ICSI with insufficient vitamin D levels, regardless of the initial outcome. The primary focus of this study was to evaluate the effect of vitamin D levels on implantation, with secondary outcomes exploring the associations between vitamin D levels and clinical pregnancy and live birth rates.

Materials and Methods

Study Design

This quasi-experimental study was conducted at the Libyan National Fertility Centre between 2019 and 2020. The study involved sixty infertile women who had previously undergone an ICSI cycle (and had a deficiency in vitamin D levels). Following the correction of vitamin D levels in these women, a second ICSI cycle was performed. The primary outcomes focused on positive pregnancy tests, whereas secondary outcomes encompassed clinical pregnancy rates (fetal heart positivity by the 7th week of gestation) (9), live birth rates, and oocyte quality. Vitamin D levels were defined as insufficient if < 20 ng/ml and sufficient if ≥ 20 ng/ml, as per the guidelines of the Endocrine Society (18).

Considering the heterogeneity observed in previous studies, potential confounding factors were addressed to minimize bias. Patients with polycystic ovaries, endometriosis, premature ovarian insufficiency, and male factor infertility were excluded. In addition, confounding factors such as age, type of infertility, ovarian reserve markers, hormones affecting ovulation (thyroid-stimulating hormone and prolactin), ovarian stimulation protocol, oocyte and embryo qualities, endometrial thickness, and pregnancy rate with its outcome were assessed.

Study Population and Data Collection

Patient files were scrutinized, and data were meticulously recorded in a Microsoft Excel spreadsheet. The collected data comprised four main categories: demographic data (including patients' characteristics), hormones affecting ovulation (TSH, prolactin), ovarian stimulation characteristics (type of protocol, stimulated drug details, starting dose, total dose, and treatment duration), and embryological and ovarian stimulation outcome characteristics (type of assisted reproductive technology treatment, number of follicles aspirated, number of retrieved and quality of oocytes, maturation rate, fertilization rate, number of embryo transfers, day of transfer, and quality of transferred embryos). Pregnancy outcomes, such as biochemical pregnancy, miscarriage rate, clinical pregnancy, ongoing pregnancy, and live birth, were also documented.

Eligible participants under 40 years of age had undergone one ICSI cycle involving fresh embryo transfer with insufficient vitamin D levels and diagnostic hysteroscopy. These patients received at least three months of vitamin D treatment (injection and/or oral therapy). Plasma vitamin D levels were measured using the Elecsys vitamin D Total immunoassay on a Cobas6000 immuno-analyzer (Roche Diagnostics, Mannheim, Germany). Following the correction of vitamin D levels, another ICSI cycle was performed. Basal hormone levels (FSH, LH, AMH, and estradiol) were assessed on day 2 of the menstrual cycle. AFC was measured using transvaginal ultrasonography on day 2 of the menstrual cycle.

Statistical Analysis

Statistical analysis of the data from this study was conducted using SPSS software, Version 20.0 (SPSS version 20, USA). Categorical data are delineated as percentages, whereas continuous variables are expressed as mean ± standard deviation (M±SD). The comparison between the two groups employed the student's t-test for continuous variables and the chi-square test for nominal variables. The *p*-value of <.05 was deemed indicative of statistical significance.

Results

The study encompassed a cohort of sixty patients, and an overview of patients' demographic characteristics is detailed in Table 1.

The average age among participants with insufficient vitamin D was 32.75 ± 4.73, while during the ICSI cycle with sufficient vitamin D, it was 32.97 ± 4.68. Regarding the duration of infertility, individuals with insufficient vitamin D exhibited a mean duration of 4.0 ± 2.5 years, whereas those with sufficient vitamin D had a mean duration of 5.1 ± 2.5 years.

Table 1: The age and duration (Y) of infertility

Variable	Insufficient vitamin D (n=60) Mean ± SD	Sufficient vitamin D (n=60) Mean ± SD
Age (years)	32.75 ± 4.73	32.97± 4.68
Duration of Infertility (years)	4.0 ± 2.5	5.1 ± 2.5

Table 2 presents the mean ± standard deviation (SD) values for FSH, LH, E2, TSH, Prolactin, and vitamin D levels in individuals categorized by insufficient and sufficient vitamin D status. The study population was stratified into two groups based on vitamin D levels—insufficient and sufficient. Clinical pregnancy rates were 31.67% and 61.67% in the insufficient and sufficient vitamin D groups, respectively (*p*=.016). The live birth rate was also significantly higher in the sufficient vitamin D status group (*p*<.001). Furthermore, the abortion rate was observed to be lower in the sufficient vitamin D status group compared to those with insufficient vitamin D levels (Table 3).

The analysis revealed a positive impact of sufficient vitamin D levels on the configuration of endometrial thickness and pattern between the two groups (*p*=.027). However, no statistically significant difference in oocyte quality was observed between the two groups (Table 4).

Table 2: **Hormonal profile**

Variable	Insufficient vitamin D (n=60) Mean ± SD	Sufficient vitamin D (n=60) Mean ± SD
FSH	6.94 ± 1.92	6.58 ± 2.40
LH	4.71 ± 1.78	4.99 ± 1.96
E2	33.00 ± 7.46	34.34 ± 8.97
TSH	1.85 ± 0.77	2.04 ± 0.91
Prolactin	13.15 ± 8.31	13.67 ± 8.77
Vitamin D level	9.78 ± 5.53	44.16 ± 17.27

Table 3: **Clinical pregnancy rate, live birth rate, and abortion rate between two groups**

Variable	Insufficient vitamin D (n=60)	Sufficient vitamin D (n=60)	*p*-value
Clinical pregnancy rate CPR	19 (31.67%)	37 (61.67%)	.016
Live birth rate LBR	9 (47.37%)	31 (83.78%)	.001
Abortion rate	10 (52.63%)	6 (16.22%)	.317

p: p-value for comparing between the two studied groups. Statistically significant at $p<.05$.

Table 4: **Endometrial pattern, thickness, and oocyte quality between two groups**

Variable	Insufficient vitamin D (n=60)	Sufficient vitamin D (n=60)	*p*-value
Endometrial thickness on the day of hCG administration (cm)	1.14 ± 1.36	1.0 ± 1.5	.065
Endometrial pattern (healthy, triple line)	31 (51.67%)	51 (85%)	.027
Oocytes quality (mii)	34 (56.67%)	51 (85%)	.065
Embryo grade a transfer	21 (35%)	36 (60%)	.047
Blastocysts transfer	7 (11.67%)	13(21.67%)	.180

p: p-value for comparing between the two studied groups. Statistically significant at $p<.05$.

Discussion

This quasi-experimental study systematically explored the impact of vitamin D insufficiency on implantation rates and pregnancy outcomes in infertile women undergoing ICSI cycles. The study findings indicate a favorable association between sufficient vitamin D levels and positive outcomes, including increased clinical pregnancy rates, improved endometrial thickness and pattern, and higher live birth rates than those with insufficient vitamin D levels.

This study meticulously controlled for confounding variables by excluding patients with conditions such as polycystic ovaries, endometriosis, premature ovarian failure, and male factor infertility. Enrolment of a uniform population, comprising both cases and controls, enhances the precision of the study results.

The study's observations align with some prior research, such as the study by Garbedian et al., classified the population according to the level of vitamin D into three groups: deficiency with level <25 nmol/L, insufficient involved 25–74 nmol/L and sufficiency groups with ≥75 nmol/L and the results was 1.2%, 53.8%, and 45.1%, respectively (19). They also reported a higher likelihood of clinical pregnancy in women with sufficient vitamin D levels (18-19). Consistent with the existing literature, this study supports the positive correlation between vitamin D levels and clinical pregnancy and live birth rates (5,20- 22).

However, conflicting results have been reported in other studies that did not find a significant role for vitamin D in ICSI outcomes, with some studies challenging the recommendation for vitamin D supplementation before ICSI (6,16,23). Conflicting outcomes were subjected to multivariate logistic regression analysis during the statistical examination, revealing that vitamin D status did not correlate significantly with pregnancy outcomes. Consequently, these findings do not support advocating for routine measurement of vitamin D levels in the context of infertility. In addition, specific investigations employing both serum and follicular fluid assessments for vitamin D reported a lack of substantial association between pregnancy rates and serum or follicular vitamin D levels. It is essential to note that study conclusions may arise from methodological differences, study design, and population characteristics (5).

Limitations of the study

This study acknowledges certain limitations, primarily the modest sample size, which may impact the generalizability of the results. Future research endeavors with larger sample sizes are recommended to enhance the generalizability of the results. Additionally, the study suggests a need to measure vitamin D levels in follicular fluid and serum levels to assess its impact on oocyte quality comprehensively.

Strengths and Implications of this Study

This prospective cohort study brings valuable strengths to the forefront by employing a robust design that systematically explores the impact of vitamin D levels on ICSI outcomes. By adopting a comprehensive approach to data collection and excluding potential confounding factors, this study enhances its internal validity and provides a nuanced understanding of the subject. The positive correlation between sufficient vitamin D levels and improved ICSI outcomes has significant implications for clinical practice. These findings suggest that assessing and correcting vitamin D levels could be a valuable intervention in infertility treatment protocols, guiding clinical decision-making and potentially improving patient outcomes. Furthermore, this study contributes to the existing body of literature, offering insights that can inform future research endeavors and prompting a deeper exploration of the mechanisms underlying the association between vitamin D and reproductive outcomes. Overall, the study's strengths lie in its design, data collection approach, and potential to influence clinical practices, patient counseling, and further research in assisted reproductive technologies.

Conclusion

In conclusion, this study substantiates that vitamin D levels are a positive predictive indicator of ICSI outcomes. Infertile women with sufficient vitamin D levels exhibit significantly higher positive pregnancy and live birth rates than those with lower serum vitamin D levels. From a cost-effective standpoint, the study advocates vitamin D supplementation among infertile patients to enhance clinical pregnancy and live birth rates. Furthermore, it underscores the importance of incorporating vitamin D status as a routine component of infertility workups before initiating ICSI cycles.

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