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Original Research

The relationship between vitamin D and IVF: a systematic review and meta-analysis

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Summary

Vitamin D deficiency does not influence IVF outcomes, but is related to lower pregnancy and it is important to provide more reliable evidence on vitamin D in IVF due o the published studies which are strongly contradictory. *Objectives:* To explore the effect of vitamin D in IVF with a focus on the outcome of clinical pregnancy in published studies. *Materials and Methods:* The authors used PubMed, EMBASE, Web of knowledge (SCI), and Cochrane Library Methods to explore all studies that evaluate vitamin D levels and IVF outcomes until 2016. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated for analyses. *Result:* The authors first classified two groups to investigate the influence of vitamin D in IVF. There was no significant difference with deficient vitamin D and IVF outcome (RR 0.88, 95% CI 0.74-1.04; I2 63%, seven trials; 1,865 participants). In addition, vitamin D deficiency also di not show a significant difference in IVF outcome (RR 0.91, 95 % CI 0.77–1.18, RR 0.91, 95% CI, 0.77-1.18; I2, 72 trials, 1,172 participants). *Conclusion:* There is no significance between vitamin D status (deficiency or replete) and IVF outcomes, but it was also found that vitamin D deficiency was inclined to lower IVF pregnancy outcome.

Key words: Vitamin D deficient; vitamin D replete; IVF; Clinical pregnancy rate; Endometrium; Infertility; Embryo transfer; Cut-off values.

Introduction

Vitamin D receptors (VDR) are expressed in different tissues, such as skeleton, brain, breast, pancreas, parathyroid glands, immune cells, cardiomyocytes, and reproductive organs, such as ovary, uterus, placenta, testis, hypothalamus, and pituitary [1]. Vitamin D regulates numerous physiologic processes through VDR [2]. Therefore vitamin D plays its importance in bone health, but also in the risk of chronic diseases [3].

Emerging evidence shows that an epidemic of vitamin D deficiency in our population [4]. Vitamin D deficiency may cause a wide range of effects in psychological disorders [5]. The relation between vitamin D and fertility has been investigated recently. Accordingly, in rodent models of rats in vitamin D deficiency, fertility is severely impaired. It was observed that gonadal insufficiencies, uterine hypoplasia , and impaired folliculogenesis in VDR was null in mutant female mice [6]. VDR is expressed in most reproductive organs, for example ovaries, fallopian tubes, and endometrium of the uterus [7].

The impact of vitamin D and fertility has only been investigated in recent years. The relation of vitamin D status with IVF has been investigated by a large number of studies, but remains unknown. Several studies recently reported that this vitamin D could possibly predict pregnancy success following IVF [8-10]. Some observations are supported by data that vitamin D might provide a mechanism which influences fetal-placental development [11]. Vigano *et al.* [12] studied the expression, localization, and regulation of the vitamin D system located in early pregnant endometrium. Nonetheless, up to date, results from other studies are strongly contradictory, which supporting that vitamin D does not affect the final clinical pregnancy rate [13, 14]. Taking into account the published literature, the objective of this systematic review and meta-analysis was to explore the effect of vitamin D on IVF.

Materials and Methods

The authors searched PubMed, EMBASE, Web of knowledge (SCI), and Cochrane Library. Medical Subject Headings (MeSH) included studies of Vitamin D (‘Vitamin D’, ‘25 (OH) D’ and ‘1,25 (OH)2 D’) and the other studies of IVF and ICSI (‘in vitro fertilization’, ‘assisted reproduction’, ‘infertility’ and ‘embryo transfer’). The authors used ‘AND’ to connect the two parts.

1) The target population was women undergoing assisted reproduction technologies (ART), 2) literature published in any language, 3) the association of vitamin D status and IVF/intracytoplasmatic sperm injection (ICSI) cycle was assessed, 4) vitamin D in follicular fluid or serum was included, and 5) the outcome included clinical pregnancy. Abstracts of scientific meetings, reviews, and meta-analyses were not included. Review Manager 5.0 (The Nordic Cochrane Centre, Copenhagen, Denmark) was used for meta-analysis.The results were expressed as relative

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Table 1. — *Basal characteristics of studies about vitamin D level on IVF outcomes.*

Author (year)	Location	Study	Care	Ranges	vitaminD collect	Outcome
Franasiak (2015) [17]	USA	retrospective cohort	chromosome screening	<20 20-29.9 >30	Ovulation trigger	CRP ongoing, chemical
Rudick B (2012) [9]	USA	Retrospective cohort	None	<20 20-29.9 >30	hCG administration	Live birth rate, CRP implantation rate
Fabris A (2014) [18]	Spain	Retrospective study	Fresh oocyte donation	<20 20-29.9 >30	Ovulation trigger	Implantation rate, CRP ongoing pregnancy rate
Rudick BJ (2014) [19]	USA	Retrospective cohort	Donor-recipient	<20 20-29.9 >30	Down-regulation	CRP, live-birth rate
Anifandis GM [10] (2010)	Greece	Prospective observational study	None	<20 20-29.9 >30	Oocyte retrieval	CRP
Polyzos NP (2014)[14]	Brussels	Retrospectively	Single embryo, blastocyst stage	<20 ≥20	Ovulation trigger	CRP, implantation rate
Paffoni(2014)[15]	Italy	Prospective cross-sectional study	None	<20 ≥20	Prior to COH	CRP, implantation rate

risks (RR) with 95% confidence interval (CI). Heterogeneity of treatment effects was evaluated as I^2. The random effect model was applied in $I^2 > 50\%$.

Results

The relevant 127 articles were screened in the authors' initial search. The reviews and the classification of vitamin D status not consistent in the search were excluded. Also, insufficient data were also excluded [15]. Garbedian *et al.* [16] classified serum levels of 25(OH)D as having sufficient (> 75 nmol/L) or insufficient (or deficient < 75 nmol/L). The classification was not consistent with this study, hence itwas excluded. In finally, seven studies were selected for the present analysis.

The characteristics of the included studies are summarized in Table 1. Seven studies reagrding vitamin D level on IVF outcomes were analyzed in this review. Five were retrospective cohort studies [9, 14, 17-19] and the other two were prospective cohort studies [10, 15]. In the included studies, the time of vitamin D collection was different. Three occurred on the day of ovulation trigger [14, 17, 18], one during hCG administration [9], one at the time of down-regulation [19], one at the time of oocyte retrieval [10], and one at the time of prior to initiation of controlled ovarian hyperstimulation (COH) [15]. Numerous studies showed that during IVF, the status of vitamin D was steady [2].

In the present study, the authors first classified two groups to investigate the influence of vitamin D in IVF. One group included deficient vitamin D correlated with IVF outcome. The other was vitamin D repletion associated with IVF outcome. There was no significant difference with deficient vitamin D and IVF outcome (RR 0.88, 95% CI 0.74-1.04; I2 63%, seven trials, 1,865 participants) (Figure 1). In addition, replete vitamin D also had no significant difference with IVF outcome (RR 0.91, 95 % CI 0.77–1.18), RR 0.91, 95% CI, 0.77-1.18; I2 72 trials, 1,172 participants) (Figure 2).

Discussion

The outcomes of the present review showed no significance between vitamin D status and IVF outcomes. Also, vitamin D replete did not increase with IVF outcomes. The outcome is consistent with the study mentioned by Lv *et al.* [20]. Thus, the review of Irani *et al.* [21] showed that 25OH-D correlated with IVF outcomes.

The present review suggests that vitamin D deficiency has no statistically influence on IVF outcomes; however, the authors found that the data (RR 0.88, 95% CI 0.74-1.04; I2 63%, seven trials, 1,865 participants) showed vitamin D deficiency was inclined to lower IVF pregnancy outcome.

There were some reasons for the distinct results of this review. In the most of included studies, the present authors found that the embryo quality differed from one another; some inclded transfer of blastocysts while others included transfer of embryos. Only in two studies [14, 17], infertile women underwent blastocyst transfer for IVF. Yet one study showed single blastocyst transfer. Others were diverse embryo transfers or not stated. Thus, the selected trials provided no exact IVF cycles. The present authors do not know whether it was the first IVF cycle or not. So the baseline pregnancy rate was a potential source of bias. The classification of vitamin D status was also a bias. The classification of vitamin D level is not consistent in many studies. Franasiak *et al.* implied that the vitamin D status had no meaningful cut-off values [17]. In other literature, the classification of vitamin D status is deficiency (< 20 ng/mL), insufficiency (20-29.9 ng/mL), and replete (> 30 ng/mL) [22, 23]. However, a study by Garbedian *et al.* clas-

Study or Subgroup	vitamin D <30ng/ml Events	vitamin D <30ng/ml Total	vitamin D >30ng/ml Events	vitamin D >30ng/ml Total	Weight	Risk Ratio M-H, Random, 95% CI
Anifandis 2010	26	80	3	21	4.7%	2.27 [0.76, 6.80]
Fabris A 2014	161	226	29	41	26.4%	1.01 [0.81, 1.25]
Franasiak 2015	295	421	64	96	28.9%	1.05 [0.90, 1.23]
Rudick B 2012	43	109	34	79	20.4%	0.92 [0.65, 1.29]
Rudick BJ 2015	25	64	26	35	19.6%	0.53 [0.37, 0.76]
Total (95% CI)		**900**		**272**	**100.0%**	**0.91 [0.71, 1.18]**
Total events	550		156			

Heterogeneity: Tau² = 0.05; Chi² = 14.53, df = 4 (P = 0.006); I² = 72%
Test for overall effect: Z = 0.68 (P = 0.50)

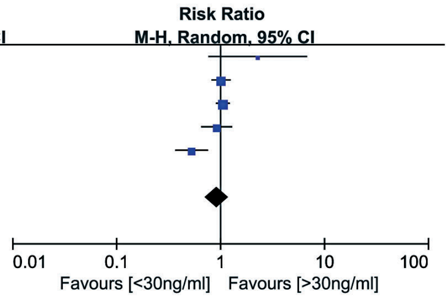

Figure 1. — The relationship between vitamin D replete and IVF.

Study or Subgroup	vitamin D <20ng/ml Events	vitamin D <20ng/ml Total	vitamin D >20ng/ml Events	vitamin D >20ng/ml Total	Weight	Risk Ratio M-H, Random, 95% CI
Anifandis 2010	10	31	19	70	5.6%	1.19 [0.63, 2.25]
Fabris A 2014	68	92	122	175	22.8%	1.06 [0.91, 1.24]
Franasiak 2015	144	206	215	311	25.0%	1.01 [0.90, 1.14]
Paffoni A2014	30	154	56	181	11.4%	0.63 [0.43, 0.93]
Polyzos 2014	98	239	70	129	19.2%	0.76 [0.61, 0.94]
Rudick B 2012	14	39	63	149	9.2%	0.85 [0.54, 1.34]
Rudick BJ 2015	9	26	42	73	6.8%	0.60 [0.34, 1.06]
Total (95% CI)		**787**		**1088**	**100.0%**	**0.88 [0.74, 1.04]**
Total events	373		587			

Heterogeneity: Tau² = 0.03; Chi² = 16.39, df = 6 (P = 0.01); I² = 63%
Test for overall effect: Z = 1.51 (P = 0.13)

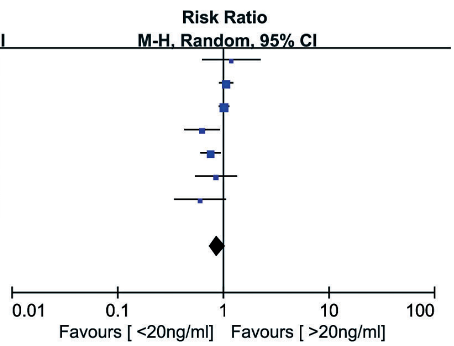

Figure 2. — The relationship between vitamin D deficiency and IVF.

sified patients as sufficient (> 75 nmol/L) or insufficient (or deficient; referred to as "insufficient": < 75 nmol/L) [16]. Thus, a Canadian guideline defined vitamin D deficiency as < 25 nmol/L, insufficiency between 25 and 74 nmol/L, and sufficiency > 75 nmol/L [24]. Lower vitamin D (< 75 nmol/L) is important for cancer and impaired immune response [25]. Vitamin D status varies from the published articles, however it was found that most of the literature adopted the classification (vitamin D status deficiency (< 20 ng/mL), insufficiency (20-29.9 ng/mL), and replete (> 30 ng/mL). In the present study, the authors defined vitamin D deficiency as below 20 ng/ml; vitamin D not deficiency (referred to as "insufficient": 20-29.9 ng/mL, and replete above 30 ng/ml).

Embryonic and endometrial factors were all important for a successful pregnancy. The embryo quality is different in the literature. Franasiak *et al.* showed the assay utilized euploid blastocysts to predict the effect of vitamin D in IVF [17]. Polyzo *et al.* [14] suggested that vitamin D deficiency has a detrimental effect on single embryo blastocyst. Thus, Rudick *et al*. [9] validated that vitamin D deficiency was not correlated with embryo quality, but had an effect on the endometrium.

HOXA10 is important for fertility and vitamin D upregulates HOXA10 expression [26]. Bagot *et al.* have recently found that HOXA10 expression in human endometrium rises dramatically at the time of embryo implantation, suggesting HOXA10 may be essential to the process [27].

The included studies were all cohorts, therefore there was a bias. The limitations of this study should be considered and in the future, more RCTs should be conducted to quantitatively evaluate existing evidence to determine serum vitamin D status in infertility populations.

The present findings showed that vitamin D deficiency did not statistically influence IVF outcomes. The outcome is consistent with the study mentioned by Lv *et al.* [20]. Aflatoonian *et al.* [28] showed that vitamin D insufficiency treatment is not associated with higher pregnancy rate. However the present authors also suggested that vitamin D deficiency was inclined to lower IVF pregnancy outcome. This is the first time that vitamin D replete was shown to not increase clinical pregnancy rate. The cut-off value of vitamin D was otherwise not in accordance with the published literatures, therefore serum vitamin D status and the cut-off values of vitamin D in infertility populations need to be further assessed.

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