Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis
Chu, Justin; Gallos, Ioannis; Tobias, Aurelio; Tan, Bee; Eapen, Abey; Coomarasamy, Aravinthan

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Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis

RUNNING TITLE
Vitamin D and assisted reproductive treatment outcome

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ABSTRACT

Study question: Is serum vitamin D associated with live birth rates in women undergoing assisted reproductive treatment?

Summary answer: Women undergoing assisted reproductive treatment who are replete in vitamin D have a higher live birth rate than women who are vitamin D deficient or insufficient.

What is known already: Vitamin D deficiency has been associated with an increased risk of abnormal pregnancy implantation as well as obstetric complications such as pre-eclampsia and fetal growth restriction. However, the effect of vitamin D on conception and early pregnancy outcomes in couples undergoing assisted reproductive treatment is poorly understood.

Study design, size, duration: A systematic review and meta-analysis of 11 published cohort studies (including 2700 women) investigating the association between vitamin D and assisted reproductive treatment outcomes.

Participants/materials, settings, methods: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and assisted reproductive treatment outcomes. Databases searched included MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL. Eleven studies matched the inclusion criteria.

Main results and the role of chance: Live birth was reported in seven of the included studies (including 2026 patients). Live birth was found to be more likely in women replete in vitamin D when compared to women with deficient or insufficient vitamin D status (OR 1.33 [1.08 to 1.65]). Five studies (including 1700 patients) found that women replete in vitamin D were more likely to achieve
a positive pregnancy test than women deficient or insufficient in vitamin D (OR 1.34 [1.04 to 1.73]).

All 11 of the included studies (including 2700 patients) reported clinical pregnancy as an outcome.

Clinical pregnancy was found to be more likely in women replete in vitamin D (OR 1.46 [1.05 to 2.02]). Six studies (including 1635 patients) reported miscarriage by vitamin D concentrations. There was no association found between miscarriage and vitamin D concentrations (OR 1.12 [0.81 to 1.54]).

The included studies scored well on the Newcastle Ottawa quality assessment scale.

Limitations, reasons for caution: Although strict inclusion criteria were used in the conduct of the systematic review, the included studies are heterogeneous in population characteristics and fertility treatment protocols.

Wider implications of the findings: The findings of this systematic review show that there is an association between vitamin D status and reproductive treatment outcomes achieved in women undergoing assisted reproductive treatment. Our results show that vitamin D deficiency and insufficiency could be important conditions to treat in women considering assisted reproductive treatments. A randomised controlled trial to investigate the benefits of vitamin D deficiency treatment should be considered to test this hypothesis.

Study funding/competing interests: No external funding was either sought or obtained for this study. The authors have no competing interests to declare.

Registration number: N/A

Key words: Vitamin D / Implantation / Assisted reproductive treatments / In vitro fertilisation / Endometrial receptivity
INTRODUCTION

Infertility causes great psychological and sometimes physical distress to one in seven couples (National Institute for Health and Care Excellence 2013). In the United Kingdom (UK), in 2014, 52,288 women underwent 67,708 in vitro fertilization (IVF) treatment cycles (Human Fertility Embryology Authority 2016). The overall success rate of these assisted reproductive treatments (ART) was 36.3% (Human Fertility Embryology Authority 2016). Since the availability of ART treatment has become more widespread, success rates have gradually increased (Grady et al. 2012). This has largely been due to the research conducted in embryology, which has enhanced our abilities to select and transfer the embryo with the highest pregnancy potential. More recently, the rate of improvement in success rates has slowed (Busso et al. 2006). There remains ample room for improvement in fertility treatments to maximize the chances of achieving pregnancy. Much of this lies in improving the likelihood for implantation of the selected embryo that is transferred into the uterus (Macklon et al. 2002).

There has been recent interest in the role of vitamin D in reproductive physiology as findings have shown that as much as 20 to 52% of women of reproductive age are deficient in vitamin D (Gordon et al. 2004; Sullivan et al. 2005; Tangpricha et al. 2002). It is postulated that vitamin D is important in the process of pregnancy implantation as vitamin D enzymes and receptors have been found in the endometrium (Lerchbaum & Rabe 2014). Additionally, vitamin D deficiency has been found to cause decreased fertility capacity, hypogonadism and uterine hypoplasia in animal studies (Halloran & DeLuca 1980; Kinuta et al. 2000; Yoshizawa et al. 1997; Panda et al. 2001). In humans, the importance of vitamin D in placental function is the most studied aspect of vitamin D in reproduction (Aghajafari et al. 2013). Specifically, vitamin D deficiency has been linked to poor placentation, leading to hypertensive disorders of pregnancy (pre-eclampsia and pregnancy induced hypertension) and fetal growth restriction (Aghajafari et al. 2013). More recently, it has been proposed that vitamin D may be a regulator of initial embryo implantation and that improper implantation, due to
vitamin D deficiency, is the cause of poor placentation (Bodnar et al. 2007; Baker et al. 2010; Robinson et al. 2011).

Our main source of vitamin D, a fat-soluble steroid hormone, is from sunlight. Only a small amount is obtained from our diet. The majority of the body’s vitamin D is in the form of vitamin D3 (cholecalciferol), which is photo-chemically synthesized in the skin (Holick 2007).

Vitamin D concentrations are usually measured by assay of serum 25-hydroxy vitamin D status. Experts in nutrition have suggested that people are at risk of the detrimental effects of vitamin D deficiency at serum 25-hydroxy vitamin D3 concentrations of less than 50 nmol/L (less than 20 ng/mL). A concentration of 50 to 75 nmol/L (21 to 29 ng/mL) is considered insufficient and greater than 75 nmol/L (greater than 30 ng/mL) is considered vitamin D replete. These vitamin D concentration cut-offs are those adopted by the Endocrine Society (Holick et al. 2011). Differing vitamin D concentration cut-offs have also been proposed by the Institute of Medicine (IOM), who suggest that vitamin D deficiency is when serum 25-hydroxy vitamin D3 concentrations are less than 30 nmol/L (less than 12 ng/mL), vitamin D insufficiency is when serum 25-hydroxy vitamin D3 concentrations are between 30 nmol/L and 50 nmol/L (between 12 and 20 ng/mL), and that serum 25-hydroxy vitamin D3 concentrations greater than 50 nmol/L (greater than 20 ng/mL) are considered replete (Ross et al. 2011). There is agreement that serum concentrations greater than 374 nmol/L (greater than 150 ng/mL) are associated with toxicity and adverse effects (Tangpricha et al. 2002; Heaney 2008; Stephanou et al. 1994; Daftary & Taylor 2006).

The biological plausibility that vitamin D plays an important role in implantation has led research groups to investigate the importance of vitamin D in patients undergoing ART. Some studies have found that replete concentrations of vitamin D lead to an increase in clinical pregnancy and live birth rates (Rudick et al. 2014; Ozkan et al. 2010; Rudick et al. 2012; Garbedian et al. 2013; Paffoni et al.)
However, others have found conflicting evidence suggesting that vitamin D has no effect on the outcome of ART (Anifandis et al. 2010; Aleyasin et al. 2011; Firouzabadi et al. 2014; Fabris et al. 2014; Franasiak et al. 2015). The aim of our review was to investigate the association between vitamin D status and reproductive outcomes by meta-analysis of the ART outcomes of published cohort studies to summarise the available evidence.

METHODS

Inclusion Criteria

The study was designed a priori with inclusion of primary articles that studied women undergoing any form of ART (IVF, ICSI and frozen embryo transfer [FET]) who had their vitamin D status checked. This could either be through blood serum or follicular fluid assay. The primary outcome was live birth rates according to vitamin D status. Secondary outcomes included biochemical pregnancy rates, and clinical pregnancy rates.

Literature search

MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL (from inception to April 2017) were searched. The search strategy used the following key words and/or medical subject heading (MeSH) terms: pregnancy, in vitro fertilization, intracytoplasmic sperm injection, assisted reproductive techniques and vitamin D. The full electronic search strategy is provided in Supplementary Table SI. References of all included primary and review articles were examined to identify relevant articles not captured by the electronic searches. No language restrictions were applied in any of the searches or study selection.
Study selection

Criteria for inclusion in the study were established prior to the literature search. Two independent reviewers (J.C. and B.T.) carried out study selection. First, the independent reviewers scrutinized the titles and abstracts of the electronic searches. Each title and abstract were included or excluded independently according to the predefined inclusion criteria; any disagreements regarding inclusion were resolved by a further reviewer (I.D.G). The full manuscripts of the titles and abstracts considered to be relevant for inclusion were obtained. When there was a duplicate publication, the most recent and complete version was selected and included. Studies that did not explicitly report results from assisted reproductive treatments according to vitamin D groups (deficient, insufficient and replete) according to Endocrine Society guidelines were excluded.

The same two independent reviewers (J.C. and B.T.) extracted the outcome data from the included studies.

Study quality assessment

Two reviewers (J.C and B.T.) used the Newcastle-Ottawa Quality Assessment Scales for observational studies to complete a quality assessment of the included manuscripts (Wells et al. 2011). The Newcastle-Ottawa scale ranges from zero to nine, awarding one star for all categories (case-cohort representative, ascertainment of exposure, outcome negative at commencement of study, outcome assessment, duration of follow up and adequacy of follow up) except comparability by design or analysis where two stars can be awarded. An arbitrary score was allocated assuming that all items have equal weighting. This was used to give a quantitative appraisal of overall quality of the individual studies. Each study received a score from each of the reviewers.
Publication Bias

Assessment for publication bias in the included studies for the outcome of clinical pregnancy was performed using Harbord’s modified test for small study effects to assess for funnel plot asymmetry (Harbord et al. 2006).

Statistical analysis

Live birth, biochemical pregnancy, clinical pregnancy and miscarriage rates were extracted from each of the included studies according to vitamin D strata. The log of the ratio and its corresponding standard error for each study was computed. Meta-analysis using inverse-variance weighting was performed to calculate the random-effects summary estimates. The square root of this number is the estimated standard deviation of the underlying effects across studies. Because we had relative measures of effect, the confidence intervals were centered on the natural logarithm of the pooled estimate and the limits exponentiated to obtain an interval on the ratio scale. Forest plots were created for each outcome, showing individual study proportions with confidence intervals (CIs) and the overall DerSimmonian-Laird pooled estimate according to vitamin D status. Heterogeneity of the treatment effects was assessed graphically with forest plots and statistically analyzed using the $\chi^2$ test. Statistical analyses were performed using Stata 12.1 (StataCorp, College Station, TX).

RESULTS

The PRISMA flow diagram (Liberati et al. 2009; Moher et al. 2009) of the review process is presented in Fig. 1. The search strategy yielded 4615 citations, of which 4505 citations were excluded as it was clear from scrutinizing the title and abstract that they did not fulfil the selection criteria. Full manuscripts of 110 articles were obtained. A total of 99 of these publications were excluded because 35 were reviews, 24 articles did not specify outcomes from ART, 17 articles did not specify investigating vitamin D, seven articles were conference abstracts or studies where there was no extractable data (Farzadi et al. 2015; Neville et al. 2016) (as they provided mean vitamin D concentrations of groups of women achieving clinical pregnancy and those that did not), five articles...
reported male infertility, four articles were animal studies, three were letters, two were duplicates, and one was a study protocol. Therefore, the total number of observational studies included in the review was 11.

Study characteristics

Study characteristics of the 11 included studies are presented in Table I. None of the included studies declared any conflicts of interest. The included studies varied in publication date between 2010 and 2015. All 11 included studies were cohort studies; six were retrospective and five were prospective in design. Sample sizes varied between 84 women to 517 women. Nine of the 11 included studies reported the ages of their study population. Seven studies had a mean age of below 37 years and two had a higher mean age of 40.5 and 40.9 years. Eight included studies used serum measurement of vitamin D, two used both follicular fluid and serum vitamin D (finding that there was high correlation between the follicular fluid vitamin D and serum vitamin D in their participants), and one study used follicular fluid alone. Of the 11 included studies, nine studies reported ART where women had used autologous oocytes. Two reported results from women who were donor egg recipients. One study used pre-implantation genetic screening to ensure that patients had karyotypically normal embryos transferred. One study chose to only study women that underwent a single blastocyst transfer. All of the 11 included studies assayed 25-hydroxy-vitamin D. Four of the included studies assessed vitamin D before the commencement of the treatment cycle, three assessed vitamin D at the time of ovulation trigger, three assessed vitamin D at the time of oocyte retrieval, and one study assessed vitamin D just before oocyte retrieval. All of the 11 included studies used the Endocrine Society classification of vitamin D status (less than 50nmol/L deficient, 50-75nmol/L insufficient, and greater than 75nmolL replete). Six of the included studies provided adjusted odds ratios, adjusting for potential confounding factors. Of these six studies, only four provided adequate detail for potential meta-analysis of adjusted odds ratios. However, two of these
studies had adjusted for vitamin D concentration and another two studies had used differing referent groups to obtain adjusted odds ratios.

A funnel plot to test for asymmetry did not find substantial evidence of publication bias (p=0.933) (Supplementary Figure S1).

All studies scored well using the Newcastle-Ottawa Quality Assessment achieving a score between 7 and 9 (Table II).

Vitamin D deficiency prevalence

Our review found a high prevalence of vitamin D deficiency. The meta-analysed prevalence for vitamin D deficiency, insufficiency and replete were 34.6% (95% CI 32.0 to 37.4), 45.3% (95% CI 42.4 to 48.5) and 25.7% (95% CI 23.4 to 28.2%) respectively.

Live birth

Seven studies (2026 participants) reported the live births achieved by women when categorized by vitamin D (Fig. 2). Meta-analysis of the data from these studies showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. The odds ratio was 1.33 (1.08 to 1.65). The meta-analysis had low statistical heterogeneity with an $I^2$ value of 5.0% (p=0.39).

Biochemical pregnancy

Five studies (1700 participants) reported the number of women that achieved a positive pregnancy test approximately two weeks after embryo transfer for the three vitamin D categories. The odds of
biochemical pregnancy in the vitamin D deficient and insufficient population versus the vitamin D replete population are presented in Fig. 3. Meta-analysis of these five cohort studies showed a greater chance of pregnancy in the vitamin D replete group when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.34 (1.04 to 1.73). There was a low level of statistical heterogeneity with an $I^2$ value of 21.0% ($p=0.28$).

### Clinical pregnancy

All 11 studies (2700 participants) reported on clinical pregnancy rate (the presence of fetal heart approximately five weeks after embryo transfer) as an outcome (Fig. 4). Pooling of the clinical pregnancy outcomes from the 11 studies showed an improved chance of clinical pregnancy in the vitamin D replete population when compared with the vitamin D deficient and insufficient population. The vitamin D replete group was more likely to achieve clinical pregnancy when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.46 (1.05 to 2.02). The $I^2$ value for this meta-analysis was 61.0% suggesting a moderate level of statistical heterogeneity ($p=0.02$).

Data could be extracted from nine of the included studies (2082 patients) to compare the chances of clinical pregnancy by using the IOM definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Pooling of the clinical pregnancy rates from these nine studies also showed that women with a vitamin D concentration of greater than 50nmol/L were more likely to achieve a clinical pregnancy when compared to women with a vitamin D concentration of below 50nmol/L with an odds ratio of 1.38 (1.04 to 1.83) (Supplementary Figure S2).
Clinical pregnancy according to source of oocyte used

The 11 included studies were divided into two groups according to the source of the oocyte (autologous or donor) used to form the embryo for transfer (Fig. 5). Nine studies (including 2334 patients) reported fertility outcomes in infertile women receiving an autologous oocyte embryo. Clinical pregnancy was found to be more likely in women who were vitamin D replete who received an autologous oocyte embryo (OR 1.39 [1.00 to 1.93]). The $I^2$ value for this meta-analysis was 56.0% suggesting a moderate level of statistical heterogeneity ($p=0.02$).

In the two studies (including 366 patients) where women received a donor oocyte embryo, no significant difference was found when comparing the clinical pregnancy in women receiving a donor oocyte embryo who were vitamin D replete when compared to women who were vitamin D deficient or insufficient (OR 2.02 [0.44 to 9.26]). The $I^2$ value for this meta-analysis was 85.0% suggesting a considerable level of statistical heterogeneity ($p=0.009$).

Miscarriage

Six studies (1635 participants) reported on the outcome of miscarriage (Fig. 6). When the data from these six studies are pooled, the chance of miscarriage in the vitamin D replete women is similar to that of vitamin D deficient and insufficient women with an odds ratio of 1.12 (0.81 to 1.54). There was a low level of statistical heterogeneity denoted by an $I^2$ value of 0.0% ($p=0.76$).

DISCUSSION

This systematic review including 11 studies suggests that the chances of achieving a live birth, a positive pregnancy test and clinical pregnancy after ART are higher in women who are vitamin D replete when compared to those who are vitamin D deficient or insufficient. Miscarriage does not appear to be associated with vitamin D status.

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Our analysis was strengthened by a number of factors. A comprehensive search strategy was used, employing relevant research databases. Additionally, a valid data synthesis method was implemented and no language restrictions were applied. The Newcastle-Ottawa Quality Assessment Scale was used to assess the quality of the included studies. The assessment of all studies scored well on this scale, suggesting low risk of bias.

There are also weaknesses in our analysis, which mainly stem from the clinical heterogeneity of the publications that were included. Some degree of heterogeneity is to be expected due to the different geographical locations that the individual cohort studies have been conducted, leading to differing population characteristics and ART protocols used. However, this is not necessarily a disadvantage as some degree of clinical heterogeneity can increase the generalisability of the findings to wider infertility populations.

Ideally, when meta-analysing cohort studies, the adjusted odds ratios (where provided) should be meta-analysed. However, in our included studies it was infrequent for the included primary studies to have provided sufficient detail of their adjusted analysis for known confounding factors such as age and BMI. Therefore, we were unable to perform a meta-analysis of adjusted odds ratios.

One source of clinical heterogeneity between the included studies is in the timing of vitamin D assessment. Some of the studies measured their participants’ vitamin D status before the start of ART, whereas others measured vitamin D at the time of oocyte retrieval. Vitamin D status is known to not fluctuate over time unless vitamin D deficiency or insufficiency is actually treated (Anagnostis et al. 2013). Therefore, the importance of the difference in timing of the vitamin D assessment reduces.
There were also differences in the bio-fluid used to assess vitamin D status amongst the included studies. Three of the included studies measured vitamin D in the follicular fluid aspirated at the time of oocyte retrieval. The remaining studies used blood serum for vitamin D measurement. Reassuringly, a number of previously published studies have found that assays of vitamin D in follicular fluid or blood serum produce results that are highly correlative (Aleyasin et al. 2011; Anifandis et al. 2010; Firouzabadi et al. 2014; Ozkan et al. 2010). Serum vitamin D would be measured more conveniently in women undergoing ART and could be tested before the start of treatment to allow time for correction of deficiency.

We found that the likelihood of achieving a positive pregnancy test after embryo transfer was higher in women who were replete in vitamin D. This would support the hypothesis that vitamin D affects embryo implantation. Two of the included studies have tried to investigate the effect of vitamin D on implantation further by only including women undergoing oocyte recipient treatment cycles (Fabris et al. 2014; Rudick et al. 2014). Isolating recipients of donor oocyte embryos aims to reduce the impact of oocyte quality on reproductive outcomes. Donated oocytes would be sourced from younger women with higher quality oocytes and therefore implantation can be investigated more accurately. Meta-analysis of the clinical pregnancy data from these two studies (including 366 patients) did not show a statistically significant difference in chance of clinical pregnancy between the vitamin D replete and vitamin D deficient or insufficient populations. However, the data may suggest a higher chance of clinical pregnancy in the vitamin D replete group. It is likely that the failure to reach statistical significance is due to the low number of participants in view of the wide confidence intervals (Schünemann et al. 2011). Removal of these two studies from the overall analysis did not alter the overall association between vitamin D concentration and clinical pregnancy.
Seasonal variations in conception rates have been established (Rojansky et al. 1992) with higher conception rates found in the Summer and Autumn. Although many hypotheses have been postulated to explain this phenomenon (e.g. reduced ovulation rates and poorer sperm quality in darker months) the exact mechanism behind this has not been explained. It is possible that an increase in sun exposure and greater sunlight luminosity increases the body's store of vitamin D, thereby yielding higher conception rates in Summer and Autumn.

Although the debate regarding the importance of vitamin D and seasonal variation in reproductive health continues, its impact on immunomodulation within the endometrium with a resultant reduction in active inflammatory cytokines is now well understood (Holick 2007). The expression of vitamin D receptors at the level of the endometrium and the role of vitamin D in the transcription of HOX10A gene (found to be of key importance in implantation) suggest that the immunomodulatory effects of vitamin D may have a direct impact on implantation and therefore the likelihood of reproductive treatment success (Evans et al. 2004).

Ethnicity has also been found to be a prognostic marker for IVF treatment success, with women of Asian and Black ethnic origins having worse reproductive outcomes (Dhillon et al. 2016). One possible explanation for this finding could be lower serum vitamin D concentrations in these ethnic groups or differences in the vitamin D receptor gene polymorphisms (Ingles 2007; John et al. 2007).

Our review demonstrates that replete vitamin D status is associated with greater chances of ART success. This could be via the actions of vitamin D on the endometrium promoting embryo implantation or as a surrogate marker for general well-being (Lerchbaum & Rabe 2014). Vitamin D serum testing is relatively cheap and widely available and its treatment is not costly. Therefore it may be beneficial to diagnose and treat vitamin D deficiency in women planning ART to optimize their pregnancy outcomes. Correction of vitamin D deficiency in these patients would also be of
benefit during pregnancy, as replete vitamin D concentrations have been found to reduce the risk of obstetric complications such as gestational diabetes (Wang et al. 2012; Zhang et al. 2015), pre-eclampsia (Moon et al. 2015; De-Regil et al. 2012; Wei 2014), and fetal growth restriction (Conde-Agudelo et al. 2013; Khalessi et al. 2015). To further investigate the value of treatment of vitamin D deficiency in the infertile population an interventional trial would be necessary.

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Authors’ roles

JC and AC were responsible for defining the research question. JC designed the strategy for literature search. JC and BT assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by AT and IDG. AE assisted in the design of the systematic review search strategy and in manuscript preparation. JC wrote the first draft of the manuscript and is its guarantor. All authors revised it critically for important intellectual content and gave final approval of the version to be published.

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Conflicts of interest

None to declare


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Yoshizawa, T. et al., 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation,

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http://www.nature.com/ng/journal/v16/n4/abs/ng0897-391.html.

Zhang, M.X. et al., 2015. Vitamin D deficiency increases the risk of gestational diabetes mellitus: A meta-analysis of observational studies. *Nutrients*, 7(10), pp.8366–8375. Available at:
Figure Legends

Figure 1. PRISMA flow diagram for study selection.

Figure 2. Meta-analysis of studies reporting live birth by vitamin D concentrations. Meta-analysis of the data from seven included studies that reported live birth as an outcome showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. F-H, Fixed; Fixed effects (Mantel-Haenszel).

Figure 3. Meta-analysis of studies reporting biochemical pregnancy by vitamin D concentrations. Meta-analysis of the data from five included studies that reported biochemical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving a positive pregnancy test from ART when compared with women with vitamin D deficiency or insufficiency.

Figure 4. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations. Meta-analysis of the data from all 11 of the included studies that reported clinical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving clinical pregnancy from ART when compared with women with vitamin D deficiency or insufficiency.

Figure 5. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations according to source of oocyte. Meta-analysis of the data from nine included studies showed that women who are vitamin D replete have a higher chance of achieving a clinical pregnancy from ART using autologous oocytes when compared with women with vitamin D deficiency or insufficiency. Meta-analysis of the data from two included studies showed no difference in the chance of clinical pregnancy in women replete, insufficient or deficient in vitamin D undergoing ART using donor oocytes.
Figure 6. Meta-analysis of studies reporting miscarriage by vitamin D concentrations. Meta-analysis of the data from six included studies that reported miscarriage as an outcome showed no difference in the chance of miscarriage in women replete, insufficient or deficient in vitamin D undergoing ART.

Supplementary Figure S1. Vitamin D and in vitro fertilisation treatment clinical pregnancy outcomes publication bias funnel plot. The funnel plot to test for asymmetry showed no substantial evidence of publication bias.

Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs. Data could be extracted from nine of the included studies to compare the chances of clinical pregnancy by using the Institute of Medicine definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Meta-analysis of the data from these nine studies showed that women who are vitamin D replete have a higher chance of achieving clinical pregnancy from ART when compared with women with vitamin D deficiency or insufficiency according to Institute of Medicine vitamin D cut-offs.
Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis

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Vitamin D and assisted reproductive treatment outcome

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ABSTRACT

Study question: Is serum vitamin D associated with live birth rates in women undergoing assisted reproductive treatment?

Summary answer: Women undergoing assisted reproductive treatment who are replete in vitamin D have a higher live birth rate than women who are vitamin D deficient or insufficient.

What is known already: Vitamin D deficiency has been associated with an increased risk of abnormal pregnancy implantation as well as obstetric complications such as pre-eclampsia and fetal growth restriction. However, the effect of vitamin D on conception and early pregnancy outcomes in couples undergoing assisted reproductive treatment is poorly understood.

Study design, size, duration: A systematic review and meta-analysis of 11 published cohort studies (including 2700 women) investigating the association between vitamin D and assisted reproductive treatment outcomes.

Participants/materials, settings, methods: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and assisted reproductive treatment outcomes. Databases searched included MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL. Eleven studies matched the inclusion criteria.

Main results and the role of chance: Live birth was reported in seven of the included studies (including 2026 patients). Live birth was found to be more likely in women replete in vitamin D when compared to women with deficient or insufficient vitamin D status (OR 1.33 [1.08 to 1.65]). Five studies (including 1700 patients) found that women replete in vitamin D were more likely to achieve
a positive pregnancy test than women deficient or insufficient in vitamin D (OR 1.34 [1.04 to 1.73]).

All 11 of the included studies (including 2700 patients) reported clinical pregnancy as an outcome.

Clinical pregnancy was found to be more likely in women replete in vitamin D (OR 1.46[1.05 to 2.02]). Six studies (including 1635 patients) reported miscarriage by vitamin D concentrations. There was no association found between miscarriage and vitamin D concentrations (OR 1.12 [0.81 to 1.54]).

The included studies scored well on the Newcastle Ottawa quality assessment scale.

Limitations, reasons for caution: Although strict inclusion criteria were used in the conduct of the systematic review, the included studies are heterogeneous in population characteristics and fertility treatment protocols.

Wider implications of the findings: The findings of this systematic review show that there is an association between vitamin D status and reproductive treatment outcomes achieved in women undergoing assisted reproductive treatment. Our results show that vitamin D deficiency and insufficiency could be important conditions to treat in women considering assisted reproductive treatments. A randomised controlled trial to investigate the benefits of vitamin D deficiency treatment should be considered to test this hypothesis.

Study funding/competing interests: No external funding was either sought or obtained for this study. The authors have no competing interests to declare.

Registration number: N/A

Key words: Vitamin D / Implantation / Assisted reproductive treatments / In vitro fertilisation / Endometrial receptivity
INTRODUCTION

Infertility causes great psychological and sometimes physical distress to one in seven couples (National Institute for Health and Care Excellence 2013). In the United Kingdom (UK), in 2014, 52,288 women underwent 67,708 in vitro fertilization (IVF) treatment cycles (Human Fertility Embryology Authority 2016). The overall success rate of these assisted reproductive treatments (ART) was 36.3% (Human Fertility Embryology Authority 2016). Since the availability of ART treatment has become more widespread, success rates have gradually increased (Grady et al. 2012). This has largely been due to the research conducted in embryology, which has enhanced our abilities to select and transfer the embryo with the highest pregnancy potential. More recently, the rate of improvement in success rates has slowed (Busso et al. 2006). There remains ample room for improvement in fertility treatments to maximize the chances of achieving pregnancy. Much of this lies in improving the likelihood for implantation of the selected embryo that is transferred into the uterus (Macklon et al. 2002).

There has been recent interest in the role of vitamin D in reproductive physiology as findings have shown that as much as 20 to 52% of women of reproductive age are deficient in vitamin D (Gordon et al. 2004; Sullivan et al. 2005; Tangpricha et al. 2002). It is postulated that vitamin D is important in the process of pregnancy implantation as vitamin D enzymes and receptors have been found in the endometrium (Lerchbaum & Rabe 2014). Additionally, vitamin D deficiency has been found to cause decreased fertility capacity, hypogonadism and uterine hypoplasia in animal studies (Halloran & DeLuca 1980; Kinuta et al. 2000; Yoshizawa et al. 1997; Panda et al. 2001). In humans, the importance of vitamin D in placental function is the most studied aspect of vitamin D in reproduction (Aghajafari et al. 2013). Specifically, vitamin D deficiency has been linked to poor placentation, leading to hypertensive disorders of pregnancy (pre-eclampsia and pregnancy-induced hypertension) and fetal growth restriction (Aghajafari et al. 2013). More recently, it has been proposed that vitamin D may be a regulator of initial embryo implantation and that improper implantation, due to
vitamin D deficiency, is the cause of poor placentation (Bodnar et al. 2007; Baker et al. 2010; Robinson et al. 2011).

Our main source of vitamin D, a fat-soluble steroid hormone, is from sunlight. Only a small amount is obtained from our diet. The majority of the body’s vitamin D is in the form of vitamin D3 (cholecalciferol), which is photo-chemically synthesized in the skin (Holick 2007).

Vitamin D concentrations are usually measured by assay of serum 25-hydroxy vitamin D3 status. Experts in nutrition have suggested that people are at risk of the detrimental effects of vitamin D deficiency at serum 25-hydroxy vitamin D3 concentrations of less than 50 nmol/L (less than 20 ng/mL). A concentration of 50 to 75 nmol/L (21 to 29 ng/mL) is considered insufficient and greater than 75 nmol/L (greater than 30 ng/mL) is considered vitamin D replete. These vitamin D concentration cut-offs are those adopted by the Endocrine Society (Holick et al. 2011). Differing vitamin D concentration cut-offs have also been proposed by the Institute of Medicine (IOM), who suggest that vitamin D deficiency is when serum 25-hydroxy vitamin D3 concentrations are less than 30 nmol/L (less than 12 ng/mL), vitamin D insufficiency is when serum 25-hydroxy vitamin D3 concentrations are between 30 nmol/L and 50 nmol/L (between 12 and 20 ng/mL), and that serum 25-hydroxy vitamin D3 concentrations greater than 50 nmol/L (greater than 20 ng/mL) are considered replete (Ross et al. 2011). There is agreement that serum concentrations greater than 374 nmol/L (greater than 150 ng/mL) are associated with toxicity and adverse effects (Tangpricha et al. 2002; Heaney 2008; Stephanou et al. 1994; Daftary & Taylor 2006).

The biological plausibility that vitamin D plays an important role in implantation has led research groups to investigate the importance of vitamin D in patients undergoing ART. Some studies have found that replete concentrations of vitamin D lead to an increase in clinical pregnancy and live birth rates (Rudick et al. 2014; Ozkan et al. 2010; Rudick et al. 2012; Garbedian et al. 2013; Paffoni et al. 2011).
However, others have found conflicting evidence suggesting that vitamin D has no effect on the outcome of ART (Anifandis et al. 2010; Aleyasin et al. 2011; Firouzabadi et al. 2014; Fabris et al. 2014; Franasiak et al. 2015). The aim of our review was to investigate the association between vitamin D status and reproductive outcomes by meta-analysis of the ART outcomes of published cohort studies to summarise the available evidence.

METHODS

Inclusion Criteria

The study was designed a priori with inclusion of primary articles that studied women undergoing any form of ART (IVF, ICSI and frozen embryo transfer [FET]) who had their vitamin D status checked. This could either be through blood serum or follicular fluid assay. The primary outcome was live birth rates according to vitamin D status. Secondary outcomes included biochemical pregnancy rates, and clinical pregnancy rates.

Literature search

MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL (from inception to April 2017) were searched. The search strategy used the following key words and/or medical subject heading (MeSH) terms: pregnancy, in vitro fertilization, intracytoplasmic sperm injection, assisted reproductive techniques and vitamin D. The full electronic search strategy is provided in Supplementary Table SI. References of all included primary and review articles were examined to identify relevant articles not captured by the electronic searches. No language restrictions were applied in any of the searches or study selection.
Study selection

Criteria for inclusion in the study were established prior to the literature search. Two independent reviewers (J.C. and B.T.) carried out study selection. First, the independent reviewers scrutinized the titles and abstracts of the electronic searches. Each title and abstract were included or excluded independently according to the predefined inclusion criteria; any disagreements regarding inclusion were resolved by a further reviewer (I.D.G). The full manuscripts of the titles and abstracts considered to be relevant for inclusion were obtained. When there was a duplicate publication, the most recent and complete version was selected and included. Studies that did not explicitly report results from assisted reproductive treatments according to vitamin D groups (deficient, insufficient and replete) according to Endocrine Society guidelines were excluded.

The same two independent reviewers (J.C. and B.T.) extracted the outcome data from the included studies.

Study quality assessment

Two reviewers (J.C and B.T.) used the Newcastle-Ottawa Quality Assessment Scales for observational studies to complete a quality assessment of the included manuscripts (Wells et al. 2011). The Newcastle-Ottawa scale ranges from zero to nine, awarding one star for all categories (case-cohort representative, ascertainment of exposure, outcome negative at commencement of study, outcome assessment, duration of follow up and adequacy of follow up) except comparability by design or analysis where two stars can be awarded. An arbitrary score was allocated assuming that all items have equal weighting. This was used to give a quantitative appraisal of overall quality of the individual studies. Each study received a score from each of the reviewers.
Publication Bias

Assessment for publication bias in the included studies for the outcome of clinical pregnancy was performed using Harbord’s modified test for small study effects to assess for funnel plot asymmetry ((Harbord et al. 2006)).

Statistical analysis

Live birth, biochemical pregnancy, clinical pregnancy and miscarriage rates were extracted from each of the included studies according to vitamin D strata. The log of the ratio and its corresponding standard error for each study was computed. Meta-analysis using inverse-variance weighting was performed to calculate the random-effects summary estimates. The square root of this number is the estimated standard deviation of the underlying effects across studies. Because we had relative measures of effect, the confidence intervals were centered on the natural logarithm of the pooled estimate and the limits exponentiated to obtain an interval on the ratio scale. Forest plots were created for each outcome, showing individual study proportions with confidence intervals (CIs) and the overall DerSimmonian-Laird pooled estimate according to vitamin D status. Heterogeneity of the treatment effects was assessed graphically with forest plots and statistically analyzed using the $\chi^2$ test. Statistical analyses were performed using Stata 12.1 (StataCorp, College Station, TX).

RESULTS

The PRISMA flow diagram (Liberati et al. 2009; Moher et al. 2009) of the review process is presented in Fig. 1. The search strategy yielded 4615 citations, of which 4505 citations were excluded as it was clear from scrutinizing the title and abstract that they did not fulfil the selection criteria. Full manuscripts of 110 articles were obtained. A total of 99 of these publications were excluded because 35 were reviews, 24 articles did not specify outcomes from ART, 17 articles did not specify investigating vitamin D, seven articles were conference abstracts or studies where there was no extractable data (Farzadi et al. 2015; Neville et al. 2016) (as they provided mean vitamin D concentrations of groups of women achieving clinical pregnancy and those that did not), five articles
reported male infertility, four articles were animal studies, three were letters, two were duplicates, and one was a study protocol. Therefore, the total number of observational studies included in the review was 11.

Study characteristics

Study characteristics of the 11 included studies are presented in Table I. None of the included studies declared any conflicts of interest. The included studies varied in publication date between 2010 and 2015. All 11 included studies were cohort studies; six were retrospective and five were prospective in design. Sample sizes varied between 84 women to 517 women. Nine of the 11 included studies reported the ages of their study population. Seven studies had a mean age of below 37 years and two had a higher mean age of 40.5 and 40.9 years. Eight included studies used serum measurement of vitamin D, two used both follicular fluid and serum vitamin D (finding that there was high correlation between the follicular fluid vitamin D and serum vitamin D in their participants), and one study used follicular fluid alone. Of the 11 included studies, nine studies reported ART where women had used autologous oocytes. Two reported results from women who were donor egg recipients. One study used pre-implantation genetic screening to ensure that patients had karyotypically normal embryos transferred. One study chose to only study women that underwent a single blastocyst transfer. All of the 11 included studies assayed 25-hydroxy-vitamin D. Four of the included studies assessed vitamin D before the commencement of the treatment cycle, three assessed vitamin D at the time of ovulation trigger, three assessed vitamin D at the time of oocyte retrieval, and one study assessed vitamin D just before oocyte retrieval. All of the 11 included studies used the Endocrine Society classification of vitamin D status (less than 50nmol/L deficient, 50-75nmol/L insufficient, and greater than 75nmol/L replete). Six of the included studies provided adjusted odds ratios, adjusting for potential confounding factors. Of these six studies, only four provided adequate detail for potential meta-analysis of adjusted odds ratios. However, two of these
studies had adjusted for vitamin D concentration and another two studies had used differing referent groups to obtain adjusted odds ratios.

A funnel plot to test for asymmetry did not find substantial evidence of publication bias (p=0.933) (Supplementary Figure S1).

All studies scored well using the Newcastle-Ottawa Quality Assessment achieving a score between 7 and 9 (Table II).

Vitamin D deficiency prevalence

Our review found a high prevalence of vitamin D deficiency. The meta-analysed prevalence for vitamin D deficiency, insufficiency and replete were 34.6% (95% CI 32.0 to 37.4), 45.3% (95% CI 42.4 to 48.5) and 25.7% (95% CI 23.4 to 28.2%) respectively.

Live birth

Seven studies (2026 participants) reported the live births achieved by women when categorized by vitamin D (Fig. 2). Meta-analysis of the data from these studies showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. The odds ratio was 1.33 (1.08 to 1.65). The meta-analysis had low statistical heterogeneity with an I² value of 5.0% (p=0.39).

Biochemical pregnancy

Five studies (1700 participants) reported the number of women that achieved a positive pregnancy test approximately two weeks after embryo transfer for the three vitamin D categories. The odds of
biochemical pregnancy in the vitamin D deficient and insufficient population versus the vitamin D replete population are presented in Fig. 3. Meta-analysis of these five cohort studies showed a greater chance of pregnancy in the vitamin D replete group when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.34 (1.04 to 1.73). There was a low level of statistical heterogeneity with an $I^2$ value of 21.0% ($p=0.28$).

Clinical pregnancy

All 11 studies (2700 participants) reported on clinical pregnancy rate (the presence of fetal heart approximately five weeks after embryo transfer) as an outcome (Fig. 4). Pooling of the clinical pregnancy outcomes from the 11 studies showed an improved chance of clinical pregnancy in the vitamin D replete population when compared with the vitamin D deficient and insufficient population. The vitamin D replete group was more likely to achieve clinical pregnancy when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.46 (1.05 to 2.02). The $I^2$ value for this meta-analysis was 61.0% suggesting a moderate level of statistical heterogeneity ($p=0.02$).

Data could be extracted from nine of the included studies (2082 patients) to compare the chances of clinical pregnancy by using the IOM definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Pooling of the clinical pregnancy rates from these nine studies also showed that women with a vitamin D concentration of greater than 50nmol/L were more likely to achieve a clinical pregnancy when compared to women with a vitamin D concentration of below 50nmol/L with an odds ratio of 1.38 (1.04 to 1.83) (Supplementary Figure S2).
Clinical pregnancy according to source of oocyte used

The 11 included studies were divided into two groups according to the source of the oocyte (autologous or donor) used to form the embryo for transfer (Fig. 5). Nine studies (including 2334 patients) reported fertility outcomes in infertile women receiving an autologous oocyte embryo. Clinical pregnancy was found to be more likely in women who were vitamin D replete who received an autologous oocyte embryo (OR 1.39 [1.00 to 1.93]). The $I^2$ value for this meta-analysis was 56.0% suggesting a moderate level of statistical heterogeneity ($p=0.02$).

In the two studies (including 366 patients) where women received a donor oocyte embryo, no significant difference was found when comparing the clinical pregnancy in women receiving a donor oocyte embryo who were vitamin D replete when compared to women who were vitamin D deficient or insufficient (OR 2.02 [0.44 to 9.26]). The $I^2$ value for this meta-analysis was 85.0% suggesting a considerable level of statistical heterogeneity ($p=0.009$).

Miscarriage

Six studies (1635 participants) reported on the outcome of miscarriage (Fig. 6). When the data from these six studies are pooled, the chance of miscarriage in the vitamin D replete women is similar to that of vitamin D deficient and insufficient women with an odds ratio of 1.12 (0.81 to 1.54). There was a low level of statistical heterogeneity denoted by an $I^2$ value of 0.0% ($p=0.76$).

DISCUSSION

This systematic review including 11 studies suggests that the chances of achieving a live birth, a positive pregnancy test and clinical pregnancy after ART are higher in women who are vitamin D replete when compared to those who are vitamin D deficient or insufficient. Miscarriage does not appear to be associated with vitamin D status.
Our analysis was strengthened by a number of factors. A comprehensive search strategy was used, employing relevant research databases. Additionally, a valid data synthesis method was implemented and no language restrictions were applied. The Newcastle-Ottawa Quality Assessment Scale was used to assess the quality of the included studies. The assessment of all studies scored well on this scale, suggesting low risk of bias.

There are also weaknesses in our analysis, which mainly stem from the clinical heterogeneity of the publications that were included. Some degree of heterogeneity is to be expected due to the different geographical locations that the individual cohort studies have been conducted, leading to differing population characteristics and ART protocols used. However, this is not necessarily a disadvantage as some degree of clinical heterogeneity can increase the generalisability of the findings to wider infertility populations.

Ideally, when meta-analysing cohort studies, the adjusted odds ratios (where provided) should be meta-analysed. However, in our included studies it was infrequent for the included primary studies to have provided sufficient detail of their adjusted analysis for known confounding factors such as age and BMI. Therefore, we were unable to perform a meta-analysis of adjusted odds ratios.

One source of clinical heterogeneity between the included studies is in the timing of vitamin D assessment. Some of the studies measured their participants’ vitamin D status before the start of ART, whereas others measured vitamin D at the time of oocyte retrieval. Vitamin D status is known to not fluctuate over time unless vitamin D deficiency or insufficiency is actually treated (Anagnostis et al. 2013). Therefore, the importance of the difference in timing of the vitamin D assessment reduces.
There were also differences in the bio-fluid used to assess vitamin D status amongst the included studies. Three of the included studies measured vitamin D in the follicular fluid aspirated at the time of oocyte retrieval. The remaining studies used blood serum for vitamin D measurement. Reassuringly, a number of previously published studies have found that assays of vitamin D in follicular fluid or blood serum produce results that are highly correlative (Aleyasin et al. 2011; Anifandis et al. 2010; Firouzabadi et al. 2014; Ozkan et al. 2010). Serum vitamin D would be measured more conveniently in women undergoing ART and could be tested before the start of treatment to allow time for correction of deficiency.

We found that the likelihood of achieving a positive pregnancy test after embryo transfer was higher in women who were replete in vitamin D. This would support the hypothesis that vitamin D affects embryo implantation. Two of the included studies have tried to investigate the effect of vitamin D on implantation further by only including women undergoing oocyte recipient treatment cycles (Fabris et al. 2014; Rudick et al. 2014). Isolating recipients of donor oocyte embryos aims to reduce the impact of oocyte quality on reproductive outcomes. Donated oocytes would be sourced from younger women with higher quality oocytes and therefore implantation can be investigated more accurately. Meta-analysis of the clinical pregnancy data from these two studies (including 366 patients) did not show a statistically significant difference in chance of clinical pregnancy between the vitamin D replete and vitamin D deficient or insufficient populations. However, the data may suggest a trend towards a higher chance of clinical pregnancy in the vitamin D replete group. It is likely that the failure to reach statistical significance is due to the low number of participants in view of the wide confidence intervals (Schünemann et al. 2011). Removal of these two studies from the overall analysis did not alter the overall association between vitamin D concentration and clinical pregnancy.
Seasonal variations in conception rates have been established (Rojansky et al. 1992) with higher conception rates found in the Summer and Autumn. Although many hypotheses have been postulated to explain this phenomenon (e.g. reduced ovulation rates and poorer sperm quality in darker months) the exact mechanism behind this has not been explained. It is possible that an increase in sun exposure and greater sunlight luminosity increases the body’s store of vitamin D, thereby yielding higher conception rates in Summer and Autumn.

Although the debate regarding the importance of vitamin D and seasonal variation in reproductive health continues, its impact on immunomodulation within the endometrium with a resultant reduction in active inflammatory cytokines is now well understood (Holick 2007). The expression of vitamin D receptors at the level of the endometrium and the role of vitamin D in the transcription of HOX10A gene (found to be of key importance in implantation) suggest that the immunomodulatory effects of vitamin D may have a direct impact on implantation and therefore the likelihood of reproductive treatment success (Evans et al. 2004).

Ethnicity has also been found to be a prognostic marker for IVF treatment success, with women of Asian and Black ethnic origins having worse reproductive outcomes (Dhillon et al. 2016). One possible explanation for this finding could be lower serum vitamin D concentrations in these ethnic groups or differences in the vitamin D receptor gene polymorphisms (Ingles 2007; John et al. 2007).

Our review demonstrates that replete vitamin D status is associated with greater chances of ART success. This could be via the actions of vitamin D on the endometrium promoting embryo implantation or as a surrogate marker for general well-being (Lerchbaum & Rabe 2014). Vitamin D serum testing is relatively cheap and widely available and its treatment is not costly. Therefore it may be beneficial to diagnose and treat vitamin D deficiency in women planning ART to optimize their pregnancy outcomes. Correction of vitamin D deficiency in these patients would also be of
benefit during pregnancy, as replete vitamin D concentrations have been found to reduce the risk of obstetric complications such as gestational diabetes (Wang et al. 2012; Zhang et al. 2015), pre-eclampsia (Moon et al. 2015; De-Regil et al. 2012; Wei 2014), and fetal growth restriction (Conde-Agudelo et al. 2013; Khalessi et al. 2015). To further investigate the value of treatment of vitamin D deficiency in the infertile population an interventional trial would be necessary.

Acknowledgments

The authors would like to acknowledge Derick Yates (Birmingham Women’s and Children’s NHS Foundation Trust) who helped design the search strategy for the systematic review and meta-analysis.

Authors’ roles

JC and AC were responsible for defining the research question. JC designed the strategy for literature search. JC and BT assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by AT and IDG. AE assisted in the design of the systematic review search strategy and in manuscript preparation. JC wrote the first draft of the manuscript and is its guarantor. All authors revised it critically for important intellectual content and gave final approval of the version to be published.

Funding

No external funding was either sought or obtained for this study.

Conflicts of interest

None to declare
References


Harbord, R.M., Egger, M. & Sterne, J., 2006. A modified test for small study effects in meta-analyses...


Human Fertility Embryology Authority, 2016. Fertility Treatment 2014,


http://humrep.oupjournals.org


http://humrep.oupjournals.org


Yoshizawa, T. et al., 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation,

Available at:


Zhang, M.X. et al., 2015. Vitamin D deficiency increases the risk of gestational diabetes mellitus: A meta-analysis of observational studies. *Nutrients*, 7(10), pp.8366–8375. Available at:

Figure Legends

Figure 1. PRISMA flow diagram for study selection.

Figure 2. Meta-analysis of studies reporting live birth by vitamin D concentrations. Meta-analysis of the data from seven included studies that reported live birth as an outcome showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. F-H, Fixed; Fixed effects (Mantel-Haenszel)

Figure 3. Meta-analysis of studies reporting biochemical pregnancy by vitamin D concentrations. Meta-analysis of the data from five included studies that reported biochemical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving a positive pregnancy test from ART when compared with women with vitamin D deficiency or insufficiency.

Figure 4. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations. Meta-analysis of the data from all 11 of the included studies that reported clinical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving clinical pregnancy from ART when compared with women with vitamin D deficiency or insufficiency.

Figure 5. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations according to source of oocyte. Meta-analysis of the data from nine included studies showed that women who are vitamin D replete have a higher chance of achieving a clinical pregnancy from ART using autologous oocytes when compared with women with vitamin D deficiency or insufficiency. Meta-analysis of the data from two included studies showed no difference in the chance of clinical pregnancy in women replete, insufficient or deficient in vitamin D undergoing ART using donor oocytes.
Figure 6. Meta-analysis of studies reporting miscarriage by vitamin D concentrations. Meta-analysis of the data from six included studies that reported miscarriage as an outcome showed no difference in the chance of miscarriage in women replete, insufficient or deficient in vitamin D undergoing ART.

Supplementary Figure S1. Vitamin D and in vitro fertilisation treatment clinical pregnancy outcomes publication bias funnel plot. The funnel plot to test for asymmetry showed no substantial evidence of publication bias.

Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs. Data could be extracted from nine of the included studies to compare the chances of clinical pregnancy by using the Institute of Medicine definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Meta-analysis of the data from these nine studies showed that women who are vitamin D replete have a higher chance of achieving clinical pregnancy from ART when compared with women with vitamin D deficiency or insufficiency according to Institute of Medicine vitamin D cut-offs.

Comment [RC11]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

Comment [RC12]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

Comment [RC13]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.
Total number of citations retrieved from electronic searches; n=4615

Citations excluded after screening title and abstract; n=4505

Full manuscripts retrieved for detailed assessment; n=110

Articles excluded after review of full manuscripts and reasons for exclusion; n=99
- Reviews n=35
- Not specific to assisted reproductive techniques n=24
- Not specific to vitamin D n=17
- Conference abstract, no data to abstract n=7
- Male infertility n=5
- Animal studies n=4
- Letter n=3
- Duplicates n=3
- Study protocol n=1

Articles included for systematic review; n=11
### Figure 2. Meta-analysis of studies reporting live birth by vitamin D concentrations

<table>
<thead>
<tr>
<th>Study</th>
<th>Vit D replete</th>
<th>Vit D deficiency/insufficiency</th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabris 2014</td>
<td>23 Events 41 Total</td>
<td>127 Events 226 Total 12.0%</td>
<td>1.00 [0.51, 1.95]</td>
</tr>
<tr>
<td>Franasiak 2015</td>
<td>60 Events 96 Total</td>
<td>264 Events 421 Total 25.7%</td>
<td>0.99 [0.63, 1.57]</td>
</tr>
<tr>
<td>Fru 2014</td>
<td>25 Events 58 Total</td>
<td>20 Events 65 Total 7.5%</td>
<td>1.70 [0.81, 3.57]</td>
</tr>
<tr>
<td>Paffoni 2014</td>
<td>19 Events 64 Total</td>
<td>57 Events 271 Total 10.7%</td>
<td>1.59 [0.86, 2.92]</td>
</tr>
<tr>
<td>Polyzos 2014</td>
<td>61 Events 129 Total</td>
<td>139 Events 368 Total 26.6%</td>
<td>1.48 [0.99, 2.22]</td>
</tr>
<tr>
<td>Rudick 2012</td>
<td>26 Events 79 Total</td>
<td>33 Events 109 Total 13.0%</td>
<td>1.13 [0.61, 2.10]</td>
</tr>
<tr>
<td>Rudick 2014</td>
<td>20 Events 35 Total</td>
<td>21 Events 64 Total 4.5%</td>
<td>2.73 [1.17, 6.38]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>502 Total</td>
<td>1524 Total 100.0%</td>
<td>1.33 [1.08, 1.65]</td>
</tr>
</tbody>
</table>

Total events: 234 Events 661 Total

Heterogeneity: Chi² = 6.33, df = 6 (P = 0.39); I² = 5%
Test for overall effect: Z = 2.62 (P = 0.009)
Figure 3. Meta-analysis of studies reporting biochemical pregnancy by vitamin D concentrations

<table>
<thead>
<tr>
<th>Study</th>
<th>Vit D replete</th>
<th>Vit D deficiency/insufficiency</th>
<th>Odds Ratio</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
</tr>
<tr>
<td>Fabris 2014</td>
<td>25</td>
<td>41</td>
<td>145</td>
<td>226</td>
</tr>
<tr>
<td>Fransasiak 2015</td>
<td>74</td>
<td>96</td>
<td>325</td>
<td>421</td>
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<tr>
<td>Ozkan 2008</td>
<td>8</td>
<td>31</td>
<td>7</td>
<td>53</td>
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<tr>
<td>Paffoni 2014</td>
<td>25</td>
<td>64</td>
<td>70</td>
<td>271</td>
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<tr>
<td>Polyzos 2014</td>
<td>86</td>
<td>129</td>
<td>210</td>
<td>368</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>361</strong></td>
<td><em>1339</em></td>
<td><strong>100.0%</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 218/757

Heterogeneity: \( \chi^2 = 5.08, df = 4 \) (P = 0.28); \( I^2 = 21\%

Test for overall effect: \( Z = 2.23 \) (P = 0.03)
Figure 4. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Vit D replete Events</th>
<th>Total</th>
<th>Vit D deficiency/insufficiency Events</th>
<th>Total</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anifanidis 2010</td>
<td>3</td>
<td>21</td>
<td>26</td>
<td>80</td>
<td>4.5%</td>
<td>0.35 [0.09, 1.28]</td>
</tr>
<tr>
<td>Fabris 2014</td>
<td>29</td>
<td>41</td>
<td>162</td>
<td>226</td>
<td>9.0%</td>
<td>0.95 [0.46, 1.99]</td>
</tr>
<tr>
<td>Firouzabadi 2014</td>
<td>4</td>
<td>16</td>
<td>70</td>
<td>205</td>
<td>5.3%</td>
<td>0.64 [0.20, 2.07]</td>
</tr>
<tr>
<td>Franasiak 2014</td>
<td>64</td>
<td>96</td>
<td>295</td>
<td>421</td>
<td>12.2%</td>
<td>0.85 [0.53, 1.37]</td>
</tr>
<tr>
<td>Fru 2014</td>
<td>37</td>
<td>58</td>
<td>30</td>
<td>65</td>
<td>9.1%</td>
<td>2.06 [1.00, 4.24]</td>
</tr>
<tr>
<td>Garbedian 2013</td>
<td>41</td>
<td>78</td>
<td>66</td>
<td>190</td>
<td>11.4%</td>
<td>2.08 [1.22, 3.56]</td>
</tr>
<tr>
<td>Ozkan 2008</td>
<td>15</td>
<td>31</td>
<td>11</td>
<td>53</td>
<td>6.7%</td>
<td>3.58 [1.36, 9.42]</td>
</tr>
<tr>
<td>Paffoni 2014</td>
<td>23</td>
<td>64</td>
<td>63</td>
<td>271</td>
<td>10.8%</td>
<td>1.85 [1.03, 3.32]</td>
</tr>
<tr>
<td>Polyzos 2014</td>
<td>70</td>
<td>129</td>
<td>168</td>
<td>368</td>
<td>13.1%</td>
<td>1.41 [0.94, 2.11]</td>
</tr>
<tr>
<td>Rudick 2012</td>
<td>34</td>
<td>79</td>
<td>43</td>
<td>109</td>
<td>10.7%</td>
<td>1.16 [0.64, 2.09]</td>
</tr>
<tr>
<td>Rudick 2014</td>
<td>26</td>
<td>35</td>
<td>25</td>
<td>64</td>
<td>7.2%</td>
<td>4.51 [1.82, 11.19]</td>
</tr>
</tbody>
</table>

Total (95% CI)     | 648                  | 2052  | 100.0%                                | 1.46  | [1.05, 2.02] |

Total events       | 346                  | 959   |                                       |       |           |

Heterogeneity: Tau² = 0.17; Chi² = 25.73, df = 10 (P = 0.004); I² = 61%
Test for overall effect: Z = 2.24 (P = 0.02)
Figure 5. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations according to source of oocyte

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Vit D replete</th>
<th>Vit D deficiency/insufficiency</th>
<th>Odds Ratio</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
<td>M-H, Random, 95% CI</td>
</tr>
<tr>
<td>Autologous oocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anifanidis 2010</td>
<td>3</td>
<td>21</td>
<td>80</td>
<td>4.5%</td>
</tr>
<tr>
<td>Firouzabadi 2014</td>
<td>4</td>
<td>16</td>
<td>70</td>
<td>5.3%</td>
</tr>
<tr>
<td>Franasiak 2014</td>
<td>64</td>
<td>96</td>
<td>295</td>
<td>12.2%</td>
</tr>
<tr>
<td>Fru 2014</td>
<td>37</td>
<td>58</td>
<td>30</td>
<td>9.1%</td>
</tr>
<tr>
<td>Garbedian 2013</td>
<td>41</td>
<td>78</td>
<td>66</td>
<td>11.4%</td>
</tr>
<tr>
<td>Ozkan 2008</td>
<td>15</td>
<td>31</td>
<td>11</td>
<td>6.7%</td>
</tr>
<tr>
<td>Paffoni 2014</td>
<td>23</td>
<td>64</td>
<td>63</td>
<td>10.8%</td>
</tr>
<tr>
<td>Polyzos 2014</td>
<td>70</td>
<td>129</td>
<td>168</td>
<td>13.1%</td>
</tr>
<tr>
<td>Rudick 2012</td>
<td>34</td>
<td>79</td>
<td>43</td>
<td>10.7%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>572</td>
<td>1762</td>
<td>83.8%</td>
<td>1.39 [1.00, 1.93]</td>
</tr>
<tr>
<td>Total events</td>
<td>291</td>
<td>772</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: τ² = 0.13; Chi² = 18.38, df = 8 (P = 0.02); I² = 56%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 1.94 (P = 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Donor oocytes</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabris 2014</td>
<td>29</td>
<td>41</td>
<td>162</td>
<td>9.0%</td>
</tr>
<tr>
<td>Rudick 2014</td>
<td>26</td>
<td>35</td>
<td>64</td>
<td>7.2%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>76</td>
<td>290</td>
<td>16.2%</td>
<td>2.02 [0.44, 9.26]</td>
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<tr>
<td>Total events</td>
<td>55</td>
<td>187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: τ² = 1.03; Chi² = 6.79, df = 1 (P = 0.009); I² = 85%</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.91 (P = 0.36)</td>
<td></td>
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<td></td>
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</table>
Figure 6. Meta-analysis of studies reporting miscarriage by vitamin D concentrations

<table>
<thead>
<tr>
<th>Study</th>
<th>Vit D replete</th>
<th></th>
<th>Vit D deficiency/insufficiency</th>
<th></th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
<th></th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabris 2014</td>
<td>4</td>
<td>41</td>
<td>17</td>
<td>226</td>
<td>6.7%</td>
<td>1.33 [0.42, 4.17]</td>
<td></td>
</tr>
<tr>
<td>Franasiak 2015</td>
<td>14</td>
<td>96</td>
<td>61</td>
<td>421</td>
<td>27.5%</td>
<td>1.01 [0.54, 1.89]</td>
<td></td>
</tr>
<tr>
<td>Fru 2014</td>
<td>12</td>
<td>37</td>
<td>10</td>
<td>30</td>
<td>10.6%</td>
<td>0.96 [0.34, 2.67]</td>
<td></td>
</tr>
<tr>
<td>Polyzos 2014</td>
<td>25</td>
<td>129</td>
<td>69</td>
<td>368</td>
<td>41.1%</td>
<td>1.04 [0.63, 1.73]</td>
<td></td>
</tr>
<tr>
<td>Rudick 2012</td>
<td>8</td>
<td>79</td>
<td>10</td>
<td>109</td>
<td>10.7%</td>
<td>1.12 [0.42, 2.97]</td>
<td></td>
</tr>
<tr>
<td>Rudick 2014</td>
<td>6</td>
<td>35</td>
<td>4</td>
<td>64</td>
<td>3.3%</td>
<td>3.10 [0.81, 11.86]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>417</td>
<td></td>
<td>1218</td>
<td>100%</td>
<td>100.0%</td>
<td>1.12 [0.81, 1.54]</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>69</td>
<td></td>
<td>171</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 2.58, df = 5 (P = 0.76); I² = 0%

Test for overall effect: Z = 0.69 (P = 0.49)
### Table I. Characteristics of included studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study design</th>
<th>Study population</th>
<th>Age of study population</th>
<th>Bio-fluid used for vitamin D assessment</th>
<th>Timing of vitamin D assessment</th>
<th>Method of vitamin D assessment</th>
<th>Vitamin D cut-offs utilised</th>
<th>Autologous or donated oocyte</th>
<th>Summary of results</th>
<th>Confounders adjustment</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anifandis et al., (2010)</td>
<td>Prospective Cohort</td>
<td>101 women undergoing IVF in Greece</td>
<td>Not reported</td>
<td>Vitamin D in follicular fluid</td>
<td>At oocyte retrieval</td>
<td>25-OH vitamin D by electrochemiluminescence immunoassay (ECLIA)</td>
<td>Deficiency &lt;50nmol/L</td>
<td>Insufficiency 50-75nmol/L</td>
<td>Replete &gt;75nmol/L</td>
<td>Autologous</td>
<td>Clinical pregnancy (intrauterine sac seen 3-4 weeks on ultrasound scan post-HCG) 10/31 deficient group 16/49 insufficient group 3/21 replete group</td>
</tr>
<tr>
<td>Fabris et al., (2014)</td>
<td>Retrospective Cohort</td>
<td>267 women undergoing donor oocyte IVF in Spain</td>
<td>Mean age 40.5 years</td>
<td>Vitamin D in serum</td>
<td>At oocyte retrieval</td>
<td>25-OH vitamin D by enzyme-linked immunosorbent assay (ELISA)</td>
<td>Deficiency &lt;50nmol/L</td>
<td>Insufficiency 50-75nmol/L</td>
<td>Replete &gt;75nmol/L</td>
<td>Donated</td>
<td>Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 68/92 deficient group 94/134 insufficient group 29/41 replete group</td>
</tr>
<tr>
<td>Firouzabadi et al., (2014)</td>
<td>Prospective Cohort</td>
<td>221 women undergoing IVF in Iran</td>
<td>Mean age 29.2 years</td>
<td>Vitamin D in follicular fluid and serum</td>
<td>At oocyte retrieval</td>
<td>25-OH vitamin D by enzyme-linked immunosorbent assay (ELISA)</td>
<td>Deficiency &lt;25nmol/L</td>
<td>Insufficiency 25-75nmol/L</td>
<td>Replete &gt;75nmol/L</td>
<td>Autologous</td>
<td>Clinical pregnancy (intrauterine sac seen on ultrasound scan [time point defined]) 23/50 deficient group 47/155 insufficient group 4/16 replete group</td>
</tr>
<tr>
<td>Study</td>
<td>Type of Study</td>
<td>Participants</td>
<td>Mean Age (years)</td>
<td>Vitamin D Assessed at</td>
<td>Assay</td>
<td>Deficiency Criteria</td>
<td>Pregnancy Outcomes</td>
<td>Miscarriage</td>
<td>Live Birth</td>
<td>Adjustment for Factors</td>
<td>Pregnancy Rates</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>--------------</td>
<td>------------------</td>
<td>-----------------------</td>
<td>-------</td>
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<td>-------------------</td>
<td>-------------</td>
<td>-----------</td>
<td>----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Franasiak et al., (2015)</td>
<td>Retrospective cohort</td>
<td>517 women undergoing IVF with euploid blastocyst transfer in USA</td>
<td>35.0</td>
<td>At ovulation trigger injection</td>
<td>ELISA</td>
<td>&lt;50nmol/L</td>
<td>Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined])</td>
<td>144/206 deficient group, 151/215 insufficient group, 64/96 replete group</td>
<td>32/206 deficient group, 29/215 insufficient group, 14/96 replete group</td>
<td>131/206 deficient group, 133/215 insufficient group, 60/96 replete group</td>
<td>Adjustment for age, BMI, ethnicity, season, number of previous treatment cycles, number of embryos transferred</td>
</tr>
<tr>
<td>Fru et al., (2014)</td>
<td>Retrospective cohort</td>
<td>102 women undergoing IVF in USA</td>
<td>Not reported</td>
<td>Pre-cycle but not defined</td>
<td>Method not defined</td>
<td>&lt;50nmol/L</td>
<td>Clinical pregnancy (not defined)</td>
<td>6/18 deficient group, 24/47 insufficient group, 37/58 replete group</td>
<td>5/18 deficient group, 15/47 insufficient group, 25/58 replete group</td>
<td>Nil</td>
<td>Higher vitamin D concentrations correlated with increased likelihood of positive pregnancy test. Overall live birth rates highest in vitamin D replete group.</td>
</tr>
<tr>
<td>Garbedian et al., (2013)</td>
<td>Prospective Cohort</td>
<td>173 women undergoing IVF in Canada</td>
<td>34.5</td>
<td>Before oocyte retrieval</td>
<td>Method not defined</td>
<td>&lt;75nmol/L</td>
<td>Clinical pregnancy (not defined)</td>
<td>33/95 deficient and insufficient groups combined, 41/78 replete group</td>
<td>Data not provided</td>
<td>Data not provided</td>
<td>Adjustment for age, BMI, number of embryos transferred and vitamin D concentration</td>
</tr>
<tr>
<td>Ozkan et al., (2010)</td>
<td>Prospective Cohort</td>
<td>84 women undergoing IVF in Turkey</td>
<td>34.4</td>
<td>At ovulation trigger injection</td>
<td>Method not defined</td>
<td>&lt;50nmol/L</td>
<td>Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined])</td>
<td>5/23 deficient group, 6/30 insufficient group</td>
<td>Data not provided</td>
<td>Data not provided</td>
<td>Adjustment for age, BMI, ethnicity, number of embryos transferred and vitamin D concentration</td>
</tr>
<tr>
<td>Study</td>
<td>Type of Cohort</td>
<td>Number of Participants</td>
<td>Mean Age</td>
<td>Vitamin D Measurement</td>
<td>Pre-cycle but not defined</td>
<td>25-OH Vitamin D by Electrochemiluminescence Immunoassay (ECLIA)</td>
<td>Deficiency</td>
<td>Insufficiency</td>
<td>Repletion</td>
<td>Clinical Pregnancy Rate</td>
<td>Miscarriage</td>
</tr>
<tr>
<td>-------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>----------</td>
<td>-----------------------</td>
<td>---------------------------</td>
<td>---------------------------------------------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Paffoni et al. (2014)</td>
<td>Prospective cohort</td>
<td>335 women undergoing IVF in Italy</td>
<td>Mean age 36.9 years</td>
<td>Vitamin D in serum</td>
<td>Pre-cycle but not defined</td>
<td>25-OH vitamin D by electrochemiluminescence immunoassay (ECLIA)</td>
<td>Deficiency &lt;50nmol/L</td>
<td>Insufficiency 50-75nmol/L</td>
<td>Repletion &gt;75nmol/L</td>
<td>Autologous</td>
<td>Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined])</td>
</tr>
<tr>
<td>Polyzos et al. (2014)</td>
<td>Retrospective cohort</td>
<td>368 women undergoing IVF resulting in single blastocyst embryo transfer in Belgium</td>
<td>Mean age 30.6 years</td>
<td>Vitamin D in serum</td>
<td>At ovulation trigger injection</td>
<td>25-OH vitamin D by enzyme-linked immunosorbent assay (ELISA)</td>
<td>Deficiency &lt;50nmol/L</td>
<td>Insufficiency 50-75nmol/L</td>
<td>Repletion &gt;75nmol/L</td>
<td>Autologous</td>
<td>Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer)</td>
</tr>
</tbody>
</table>

Analysis suggested those with a vitamin D >75nmol/L had the highest chance of clinical pregnancy when compared with those with vitamin D deficiency or insufficiency.
<table>
<thead>
<tr>
<th>Rudick et al., (2012)</th>
<th>188 women undergoing IVF in USA</th>
<th>Mean age 36.0 years</th>
<th>Vitamin D in serum</th>
<th>Pre-cycle but not defined</th>
<th>25-OH vitamin D by enzyme-linked immunosorbent assay (ELISA)</th>
<th>Deficiency &lt;50nmol/L</th>
<th>Insufficiency 50-75nmol/L</th>
<th>Repletion &gt;75nmol/L</th>
<th>Autologous</th>
<th>Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer)</th>
<th>Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen or pregnancy loss after gestational sac seen)</th>
<th>Live birth</th>
<th>Live birth</th>
<th>Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen)</th>
<th>Live birth</th>
<th>Vitamin D deficiency associated with lower CPR in non-hispanic whites but not in Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rudick et al., (2014)</td>
<td>99 women undergoing donor oocyte IVF in USA</td>
<td>Mean age 40.9 years Range 21-39</td>
<td>Vitamin D in serum</td>
<td>Pre-cycle but not defined</td>
<td>25-OH vitamin D by enzyme-linked immunosorbent assay (ELISA)</td>
<td>Deficiency &lt;50nmol/L</td>
<td>Insufficiency 50-75nmol/L</td>
<td>Repletion &gt;75nmol/L</td>
<td>Donated</td>
<td>Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer)</td>
<td>Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen)</td>
<td>Live birth</td>
<td>Live birth</td>
<td>Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen)</td>
<td>Live birth</td>
<td>Vitamin D deficiency associated with lower CPR in non-hispanic whites but not in Asians</td>
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### Table II. Newcastle-Ottawa Scale appraisal of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Case representative</th>
<th>Control representative</th>
<th>Ascertainment of exposure</th>
<th>Outcome negative at start</th>
<th>Comparability by design or analysis</th>
<th>Outcome assessment</th>
<th>Duration of follow up</th>
<th>Adequacy of follow up</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anifandis et al., (2010)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>x</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Fabris et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Firouzabadi et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>x</td>
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<td>Franaśiak et al., (2015)</td>
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<td>8</td>
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<tr>
<td>Fru et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>x</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>7</td>
</tr>
<tr>
<td>Garbedian et al., (2013)</td>
<td>*</td>
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<td>*</td>
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<td>*</td>
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<td>9</td>
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<td>Ozkan et al., (2010)</td>
<td>*</td>
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<td>*</td>
<td>*</td>
<td>9</td>
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<tr>
<td>Paffoni et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>8</td>
</tr>
<tr>
<td>Polyzos et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>9</td>
</tr>
<tr>
<td>Rudick et al., (2012)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>x</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>7</td>
</tr>
<tr>
<td>Rudick et al., (2014)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>9</td>
</tr>
</tbody>
</table>
Supplementary Figure S1. Vitamin D and in vitro fertilisation treatment clinical pregnancy outcomes publication bias funnel plot
### Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs

<table>
<thead>
<tr>
<th>Study</th>
<th>Vit D replete</th>
<th>Vit D Insufficiency/deficient</th>
<th>Odds Ratio</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
</tr>
<tr>
<td>Anifanidis 2010</td>
<td>19</td>
<td>70</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>Fabris 2014</td>
<td>123</td>
<td>175</td>
<td>68</td>
<td>92</td>
</tr>
<tr>
<td>Franasiak 2014</td>
<td>215</td>
<td>311</td>
<td>144</td>
<td>206</td>
</tr>
<tr>
<td>Fru 2014</td>
<td>61</td>
<td>105</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Ozkan 2008</td>
<td>21</td>
<td>61</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Paffoni 2014</td>
<td>56</td>
<td>181</td>
<td>30</td>
<td>154</td>
</tr>
<tr>
<td>Polyzos 2014</td>
<td>70</td>
<td>129</td>
<td>98</td>
<td>239</td>
</tr>
<tr>
<td>Rudick 2012</td>
<td>63</td>
<td>149</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Rudick 2014</td>
<td>42</td>
<td>73</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>1254</td>
<td>828</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 670 vs. 384

Heterogeneity: $\tau^2 = 0.07$; $\chi^2 = 13.64$, df = 8 (P = 0.09); $I^2 = 41$

Test for overall effect: $Z = 2.23$ (P = 0.03)
**Supplementary Table S1**

*Full electronic search strategy: Vitamin D and Assisted Reproductive Treatment Outcomes*

<table>
<thead>
<tr>
<th>Line</th>
<th>Database</th>
<th>Search term</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Medline</td>
<td>“pregnancy”.ti,ab</td>
</tr>
<tr>
<td>2</td>
<td>Medline</td>
<td>“in vitro fertilisation”.ti,ab</td>
</tr>
<tr>
<td>3</td>
<td>Medline</td>
<td>“intracytoplasmic sperm injection”.ti,ab</td>
</tr>
<tr>
<td>4</td>
<td>Medline</td>
<td>“assisted reproductive treatment”.ti,ab</td>
</tr>
<tr>
<td>5</td>
<td>Medline</td>
<td>1 OR 2 OR 3 OR 4</td>
</tr>
<tr>
<td>6</td>
<td>Medline</td>
<td>VITAMIN D/</td>
</tr>
<tr>
<td>7</td>
<td>Medline</td>
<td>((vitamin ADJ D)).ti,ab</td>
</tr>
<tr>
<td>8</td>
<td>Medline</td>
<td>((cholecalciferol OR ergocalciferol)).ti,ab</td>
</tr>
<tr>
<td>9</td>
<td>Medline</td>
<td>6 OR 7 OR 8</td>
</tr>
<tr>
<td>10</td>
<td>Medline</td>
<td>5 AND 9</td>
</tr>
</tbody>
</table>

ti; title, ab; abstract, ADJ; adjacent
### PRISMA 2009 Checklist

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Title</td>
<td>1</td>
<td>Identify the report as a systematic review, meta-analysis, or both.</td>
<td>Page 1</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structured summary</td>
<td>2</td>
<td>Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.</td>
<td>Page 3 to 4</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rationale</td>
<td>3</td>
<td>Describe the rationale for the review in the context of what is already known.</td>
<td>Page 5 to 7</td>
</tr>
<tr>
<td>Objectives</td>
<td>4</td>
<td>Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).</td>
<td>Page 7</td>
</tr>
<tr>
<td>METHODS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol and registration</td>
<td>5</td>
<td>Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.</td>
<td>Not registered</td>
</tr>
<tr>
<td>Eligibility criteria</td>
<td>6</td>
<td>Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.</td>
<td>Page 7</td>
</tr>
<tr>
<td>Information sources</td>
<td>7</td>
<td>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.</td>
<td>Page 7</td>
</tr>
<tr>
<td>Search</td>
<td>8</td>
<td>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</td>
<td>Supplementary File S1</td>
</tr>
<tr>
<td>Study selection</td>
<td>9</td>
<td>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</td>
<td>Page 8</td>
</tr>
<tr>
<td>Data collection process</td>
<td>10</td>
<td>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</td>
<td>Page 8</td>
</tr>
<tr>
<td>Data items</td>
<td>11</td>
<td>List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.</td>
<td>Page 7</td>
</tr>
<tr>
<td>Risk of bias in individual studies</td>
<td>12</td>
<td>Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.</td>
<td>Page 8 to 9</td>
</tr>
<tr>
<td>Summary measures</td>
<td>13</td>
<td>State the principal summary measures (e.g., risk ratio, difference in means).</td>
<td>Page 7 to 9</td>
</tr>
<tr>
<td>Synthesis of results</td>
<td>14</td>
<td>Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis.</td>
<td>Page 9</td>
</tr>
</tbody>
</table>
# PRISMA 2009 Checklist

## Section/topic

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of bias across studies</td>
<td>15</td>
<td>Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).</td>
<td>Page 9 and supplementary file S2</td>
</tr>
<tr>
<td>Additional analyses</td>
<td>16</td>
<td>Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.</td>
<td>Page 7 to 9</td>
</tr>
</tbody>
</table>

## RESULTS

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study selection</td>
<td>17</td>
<td>Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.</td>
<td>Page 9 and figure 1</td>
</tr>
<tr>
<td>Study characteristics</td>
<td>18</td>
<td>For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.</td>
<td>Page 10 and table 1</td>
</tr>
<tr>
<td>Risk of bias within studies</td>
<td>19</td>
<td>Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).</td>
<td>Page 11, supplementary file S2 and table 1</td>
</tr>
<tr>
<td>Results of individual studies</td>
<td>20</td>
<td>For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.</td>
<td>Page 11 to 13, Figures 2 to 6 and Table 1</td>
</tr>
<tr>
<td>Synthesis of results</td>
<td>21</td>
<td>Present results of each meta-analysis done, including confidence intervals and measures of consistency.</td>
<td>Page 11 to 13, Figures 2 to 6</td>
</tr>
<tr>
<td>Risk of bias across studies</td>
<td>22</td>
<td>Present results of any assessment of risk of bias across studies (see Item 15).</td>
<td>Page 11 and supplementary file S2</td>
</tr>
<tr>
<td>Additional analysis</td>
<td>23</td>
<td>Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).</td>
<td>Page 12 to 13, Figure 5 and supplementary file S3</td>
</tr>
</tbody>
</table>

## DISCUSSION

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary of evidence</td>
<td>24</td>
<td>Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).</td>
<td>Page 13-14</td>
</tr>
</tbody>
</table>
## PRISMA 2009 Checklist

<table>
<thead>
<tr>
<th>Limitations</th>
<th>25</th>
<th>Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).</th>
<th>Page 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conclusions</td>
<td>26</td>
<td>Provide a general interpretation of the results in the context of other evidence, and implications for future research.</td>
<td>Page 14 to 17</td>
</tr>
</tbody>
</table>

### FUNDING

| Funding       | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | Page 18 |

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*For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).*

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